PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

DES TERMES, Monique
Brevatome
3, rue du Docteur Lancereaux
F-75008 Paris
FRANCE

Date of mailing (day/month/year)
07 March 2002 (07.03.02)

Applicant's or agent's file reference
B13345

IMPORTANT NOTIFICATION

International application No.
PCT/IB00/01161

International filing date (day/month/year)
23 August 2000 (23.08.00)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
CIS BIO INTERNATONIAL
Route Nationale 306
F-91400 Saclay
France

State of Nationality: FR
State of Residence: FR
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [X] the address [X] the nationality [X] the residence

Name and Address
SCHERING AKTIENGESELLSCHAFT
Wedding Mullerstrasse 178
13342 Berlin
Germany

State of Nationality: DE
State of Residence: DE
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
Jaime LEITAO

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 07 May 2001 (07.05.01)	
International application No. PCT/IB00/01161	**Applicant's or agent's file reference** B 13345 EE
International filing date (day/month/year) 23 August 2000 (23.08.00)	**Priority date** (day/month/year) 01 September 1999 (01.09.99)
Applicant BELLANDE, Emmanuel et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

17 March 2001 (17.03.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Zakaria EL KHODARY
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

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PATENT COOPERATION TREATY

PCT

REC'D 17 JAN 2002
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
B 13345 EE		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB00/01161	23/08/2000	01/09/1999
International Patent Classification (IPC) or national classification and IPC A61K51/06		
Applicant CIS BIO INTERNATIONAL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 9 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17/03/2001	15.01.2002
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Büttner, U Telephone No. +49 89 2399 7841

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB00/01161

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1,3-6,8-14,16-26, 30-34	as originally filed			
2,7,15,27-29	as received on	23/10/2001	with letter of	23/10/2001

Claims, No.:

2 (part),3 (part), 11 (part),12-20	as originally filed			
1,2 (part),3 (part), 4-10,11 (part)	as received on	23/10/2001	with letter of	23/10/2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-20
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-20
Industrial applicability (IA)	Yes:	Claims	1-20
	No:	Claims	

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: FR-A-2 736 834

Claims 1-20

(N)

D1 discloses a radio pharmaceutical product in the form of micro particles (p. 12, l. 1-5) comprising a polysaccharide (p. 7, l. 25-28) provided with a sequestering group and a radioactive metal (p. 3, l. 3). The sequestering group includes moieties having a formula such as: "N-C-S" (p.4, l. 9-14).

The subject matter of claim 1 of the present application differs in the selection of the radioactive metal respectively in the selection of the sequestering group.

Therefore the subject matter of claims 1-20 is considered to be novel according to Art. 33(2) PCT.

(IS)

D1 discloses a process for the manufacture of radio pharmaceutical product comprising a

- -polymer (preferably a polysaccharide)
- -a sequestering group linked to the polymer by covalent bonds
- -where the sequestering group is linked to the polymer via N and does contain a second chelating atom selected from N, S, or O
- -a radioactive metal

The manufacturing process involves the following steps:

- -reticulation of the polymer
- -fixation of the sequestering group; this step can include the following means (p. 5, l. 20-25): oxidation of the carrier material, reaction with the sequestering group
- -addition of the radioactive material

The process described by present invention mainly differs in the following features
- omission of the reticulation step
- selection of specific sequestering groups containing N **and** S

The problem to be solved by this differences may therefore be regarded as to provide a radio pharmaceutical product that shows a good pulmonary captation.
The applicant demonstrated that the problem is solved by products obtainable by the process as described in examples 1-9, 13 (see also p. 27 Table I).
Since D1 does not mention a good pulmonary captation nor does suggest the omission of the reticulation step, this effect was not predictable from D1.

However the invention as described in above mentioned examples is not reflected by the claims for the following reasons:
According to the description the reticulation of polymers with epichlorhydrin does not result in a good pulmory affinity. These polymers however are included within the wording of claims 1-20.
Moreover from the description (see e.g. p. 26, l. 9-11) it appears that the structure obtainable by the manufacturing process is essential to pulmonary captation.
Product claims 1-7, 18-20 however include a number of polysaccharides that are not obtainable by the process of the invention.
Therefore claims 1-20 do not solve the problem over the whole their breadth.

(IA)

Claims 1-7, 11-20

Claims 1-7, 11-20, meet the requirements of Art 33 (4) PCT.

Claims 7-9

For the assessment of the present claims 7-9 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VIII

Certain observations on the international application

The expression ""the metals of formulae .." as defined in claim 11 part b) is not clear according to Article 6 PCT.

The sentence "one cannot obtain durable pulmonary captation..without prior chemical transformation **contrary** to the product relating to the present invention" (p. 26 8-11) is not clear according to Article 5 PCT.

toxic for the organism, they must be able to be sterilised for example by autoclaving or by irradiation, they must be able to be labelled easily with a radioactive metal and be able to be packaged under the form of a stable labelling kit.

Prior Art

For example, the application for French brevet FR-A-2 273 516, deposited in 1975 by the PHARMACIA AKTIEBOLAG Company, resident in Sweden, describes the use of microspheres of amyl pectin reticulated by epichlorhydrin and labelled by a simple mixture with ^{99m}Tc for pulmonary perfusion scintigraphy. These particles present several inconveniences. In fact, only the hydroxyl groupings of amyl pectin used can allow this mixture labelling, and unfortunately they only form weak bonds with technetium and do not make stable labelling possible. In addition, the preparation procedure described uses many solvents and emulsifiers which are difficult to eliminate from the particles prepared. Furthermore, the exact rate of reticulation cannot be measured accurately nor controlled on this particle type.

Moreover, this document does not describe the kit compatible with routine utilisation in nuclear medicine. In fact, for an injectable preparation for humans, several manipulations such as adjunction of tin to the sterile flask, a centrifuging, a restoration of suspension, etc. are necessary, which is not compatible with sterility requirements.

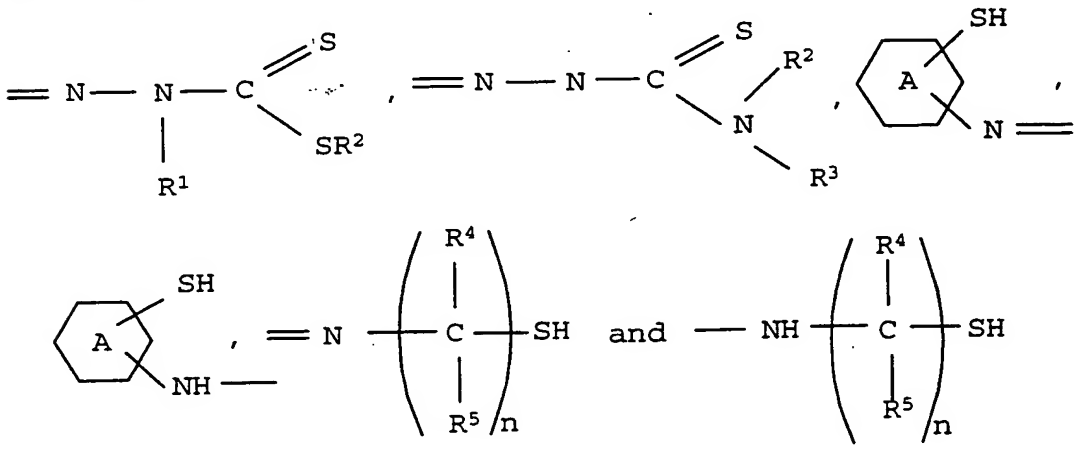
to the invention, the polysaccharide can be chosen, for example, from among natural starch, cellulose or reticulated amyl pectin.

The natural starch can, for example, be maize starch.

The polysaccharide can be in the form of microparticles, for example in the form of microspheres.

The present inventors have also demonstrated that modified cellulose according to the present invention offers very good pulmonary captation and an elimination speed slower than with starch. The modified cellulose of the present invention can therefore also be used for radiotherapy, for example with labelling with rhenium, copper, or with one of the above-mentioned metals, since it corresponds to the radiotherapy necessity of using microparticles with a longer half-life.

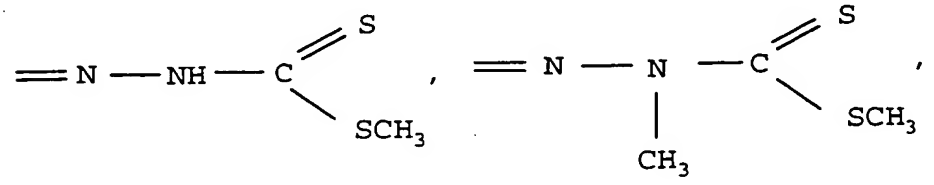
According to the invention, the sequestering groups can be chosen for example from the groups with formulae:

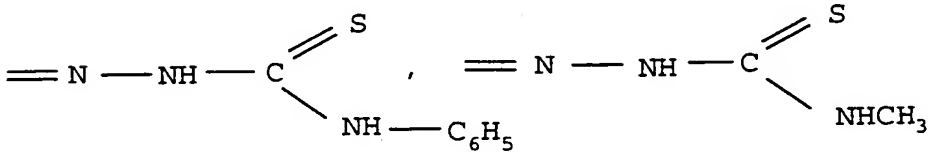


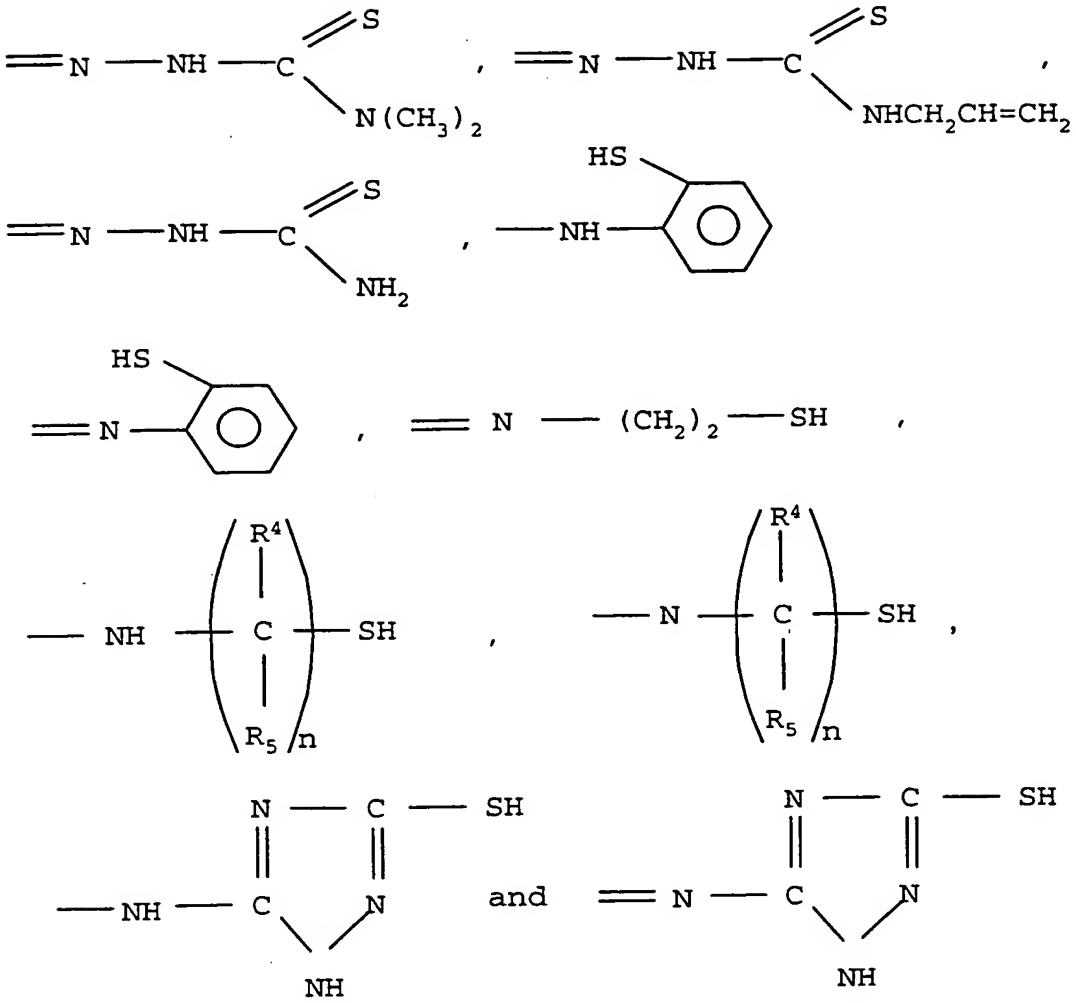
in which R^1, R^2, R^3, R^4 and R^5 are independently hydrogen atoms, saturated or unsaturated hydrocarbonic groups, carboxylic groups or aromatic groups,

is an aromatic nucleus possibly containing one or several hetero-atoms, and n is a whole number between 1 and 5.

For example, they can be chosen from among the formula groups:







According to the invention, the microparticles, for example in the shape of microspheres, can be of a dimension from 0.01 to 100 µm, preferably from 10 to 50 µm for diagnosis by pulmonary scintigraphy and from 0.1 to 5 µm for therapy.

According to the invention, the levels of sequestering groups can be from 0.1 to 50% compared to the saccharide patterns of the polysaccharide, preferably from 2 to 15%.

According to the invention, in the radiopharmaceutical product, particularly when it is

used for diagnosis, the radioactive metal can be ^{99m}Tc or gallium-67.

This can be the case, for example, when the radiopharmaceutical product is used for pulmonary scintigraphy.

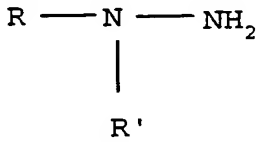
According to the invention, in the radiopharmaceutical product, in particular when it is used for therapy, the radioactive metal can be rhenium-186 or 188, copper-64 or 67, yttrium-90, erbium-169 or samarium-153.

According to the invention, said radiopharmaceutical product can be in the form of a suspension of microspheres in a physiologically acceptable liquid or in lyophilised form.

The present invention also provides a preparation procedure for the radiopharmaceutical product of the invention comprising the following stages:

(a) submit a polysaccharide, for example such as those mentioned above, to oxidation controlled by means of a periodate,

(b) react the oxidised polysaccharide with a compound containing a primary amine function or hydrazin of formula $R-NH_2$ or



(c) in which R is a hydrocarbonic or aromatic group comprising at least one atom of sulphur, in order to bond in a covalent manner to the polysaccharide with sequestering groups the metals of formulae R-NH-, R-

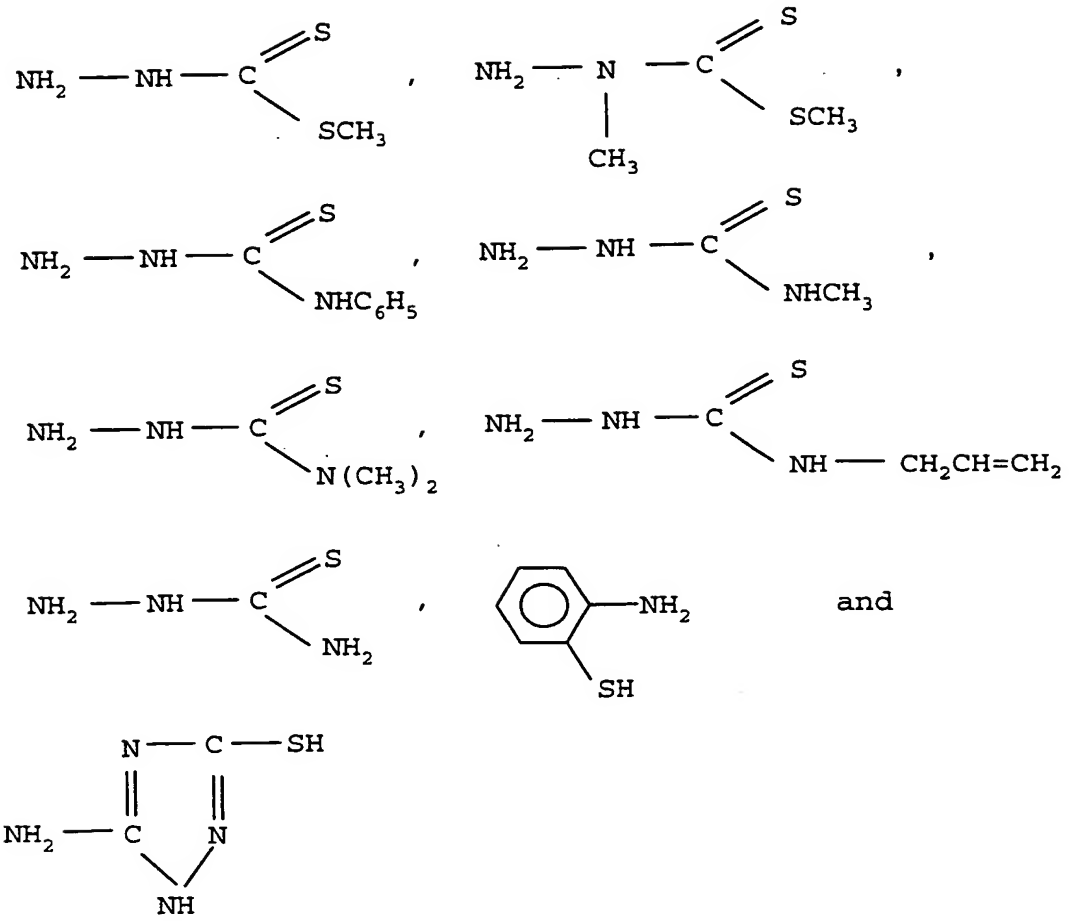
N= or R-NH-N=, and R' is a hydrogen atom or an alkyl grouping, for example methyl,

(d) react the polysaccharide comprising the sequestering groups with a salt of a radioactive metal chosen from among technetium, rhenium, copper, yttrium, erbium and samarium.

The oxidation controlled by means of a periodate can be that described, for example, in C.L. Mehltretter, "Methods in Carbohydrate Chemistry", vol. IV, 1964, applied in particular to the oxidation of starch, dextrane or cellulose. It is used in the examples given below.

According to the invention, the compound containing a primary amine function can correspond to the formula $NH_2-(CH_2)_n-SH$ with n being a whole number between 1 and 5, and can include a supplementary reducing stage of this compound with sodium borohydride between stages (b) and (c).

According to the invention, the compound bonded to the polysaccharide can correspond, for example, to one of the following formulae:

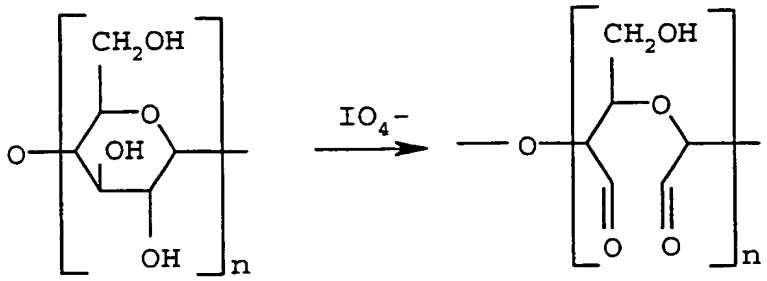


According to the invention, the level of sequestering groups fixed on the polysaccharide can be regulated by controlling the level of oxidation of the polysaccharide in stage (a) mentioned above. This oxidation level of polysaccharide can, for example, be between 10 to 50%. The level of the sequestering group can, for example, be between 2 and 15%.

In order to allow labelling of the polysaccharide according to the invention, for example with ^{99m}Tc, the inventors have therefore used a two-stage transformation method.

This method can be presented as consisting of carrying out a controlled oxidation of the

polysaccharide by the periodate in the first stage. Each unit of oxidised glucose thus generates two aldehyde groups in neighbouring positions following the chemical reaction diagram given below:

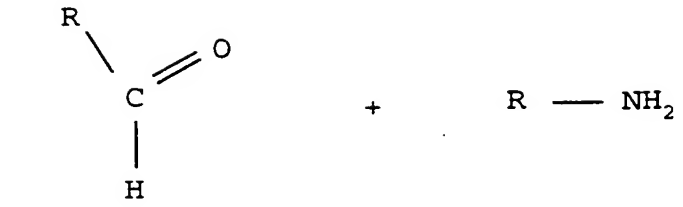


natural glucoside monomer oxidised glucoside monomer

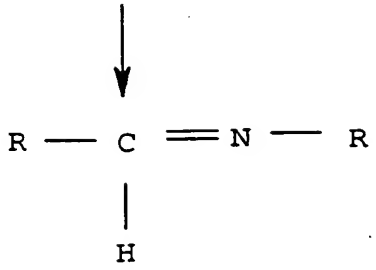
The level of oxidation of the polysaccharide can be variable and easily adjusted. In fact, the yield of this oxidation reaction is close to 100% and the level of oxidaton can be calculated from the quantities of periodate added. In general, oxidation levels lower than 50% are used so as to modify the structure of the macromolecule only slightly. The real oxidation level, ranging from 1 to 100%, can easily be determined by a colorometric method.

In the second stage, the oxidised polysaccharide is made to react with a molecule containing an amine or hydrazin grouping with the general formula RNH_2 or $RNHNH_2$ to form a chelating grouping able to sequester technetium. Thus one obtains a Schiff base type ligand or thiosemicarbazone.

This second stage can be summarised as follows:



aldehyde grouping + amine or hydrazin



functionalised aldehyde grouping

with:

1. $R=NR_1(C=S)SR_2$ (Schiff bases issued from dithiocarbazate
2. $R=NR_1(C=S)NR_2R_3$ (thiosemicarbazones)
3. R= aromatic grouping (aromatic Schiff bases)
4. R= alkyl grouping (alkylic Schiff bases); in this case the Schiff bases are not stable and one carries out a second reduction stage of the C=N bond with borohydride so as to stabilise it and then an amine C-NHR bond is obtained.

According to the invention, stage (c) can for example consist of putting into contact the microspheres of polysaccharide comprising the sequestering groups for example with a solution of pertechnetate $^{99m}TcO_4^-$ in the presence of a reducing agent, for example stannous chloride.

According to the invention, microparticles, for example microspheres, for example maize starch or

starch with a base of reticulated amyl pectin can thus be oxidised, then coupled to a molecule containing an amine or hydrazin function, for example S-methyl dithiocarbazate. These particles modified in this way can easily be labelled with, for example, ^{99m}Tc.

The present invention thus provides in particular microparticles prepared for example from a base of starch particles, which therefore do not present the inconveniences of the albumin mentioned above. In addition, the starch is described as an excipient in the pharmacopoeia. It is therefore easily available and at low cost.

The microparticles of the present invention also have the advantage of being able to be sterilised easily, for example by irradiation, and to be processed under the form of a kit ready for labelling.

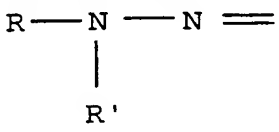
Moreover, the present inventors have demonstrated according to the present invention that the speed of pulmonary clearance can be modified according to the level of oxidation of the microparticles used in the present invention, which is not possible, for example, with human albumin microspheres.

Another advantage of the present invention lies in the simplicity of operation of the procedure: the reaction conditions being very gentle: reactions at ambient temperature, in an aqueous medium, quasi-quantitative yields. In addition, the sequestering reactions, for example with technetium, are quantitative; they take place at room temperature and without final purification which makes it possible to adapt to the requirements of sterility and simplicity

of preparation necessary for the utilisation of technetised kits in the hospital environment.

The present invention also provides a diagnosis kit which can be used, for example, for pulmonary scintigraphy. This kit comprises:

a first flask containing a polysaccharide according to the invention, that is to say provided with sequestering groups linked to the polysaccharide by covalent bonds and chosen among the groups of formulae R-NH-, R-N= and



in which R is a hydrocarbonic or aromatic group comprising at least one atom of sulphur, and in which R' is a hydrogen atom or an alkyl grouping such as methyl.

According to the invention, the polysaccharide can, for example, be in the form of microparticles, for example in the shape of microspheres, the microparticles being able to be in lyophilised form or in suspension in a pharmaceutically acceptable liquid.

The kit of the present invention can furthermore comprise a second flask containing stannous chloride preferably uin lyophilised form, or also when the polysaccharide is in lyophilised form, for example in the form of microparticles, in the first flask, said first flask can besides contain lyophilised stannous chloride.

The kits of the present invention are stable for at least twelve months as demonstrated in the examples given below.

The radiopharmaceutical product of the present invention therefore presents all the qualities required for a use such as radiopharmaceutical usage, for example for scintigraphy of pulmonary perfusion or for radiotherapy.

Other advantages will also appear when reading the following examples related to the present invention.

EXAMPLES

Example 1

A suspension of 10 g of maize starch from the pharmacopoeia is prepared, previously sieved between 10 and 40 µm, containing about 10% water, that is 0.055 mole of glucose in 100 ml of water. One adds 0.0168 mole of sodium periodate (0.3 eq) ($NaIO_4$), that is 3.6 g, dissolved in 100 ml of water. The suspension is then stirred for 18 hours at room temperature. The solution is filtered and the oxidised starch is rinsed by 5 times 20 ml of water and then 2 times 50 ml of acetone. The starch is vacuum dried and one obtains 10 g of starch oxidised at 30% (yield = 100%).

A suspension of 10 g of starch oxidised at 30% is prepared in 60 ml of a water/ethanol mixture 2/1 by volume. Next one adds 0.1 eq (0.1x0.055x2) that is 0.011 mole of S-methyl dithiocarbazate ($NH_2NH(C=S)SCH_3$), M=122, that is 1.34 g, dissolved in 10 ml of ethanol. The suspension is stirred for 18 hours at room temperature. The solution is next filtered and the

modified starch is washed by 3 times 20 ml of ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 5.4%, which corresponds to a coupling level of S-methyl dithiocarbazate (DTCZ) of 7% (7 units dithiocarbazate for 100 theoretical aldehyde functions that is 14 dithiocarbazate units for 100 glucose units). The coupling yield is therefore 70%. One thus obtains 10 g of starch oxidised at 30% and coupled to the DTCZ at 7%.

Labelling reaction with ^{99m}Tc

10 mg of starch thus modified are introduced into a flask of the penicillin type. One then adds 4 ml of physiological serum then 10 µg of $SnCL_2$, $2H_2O$ (20 µl of a solution at 0.5 mg/ml in HCl 0.1 N). Then 1 ml of a solution of $^{99m}TcO_4^-$ (5mc) is added. The solution is stirred for 15 minutes and the radiochemical purity control (RCP) is carried out by filtration of 1 ml of the solution on a Millipore filter of 0.22 µm then the filter is rinsed by 2ml of physiological serum. The labelled microspheres are retained on the filter contrary to the radioactive impurities not linked to the microspheres which are found in the filtrate. The radiochemical purity corresponds to:

RCP= (activity on the filter/total activity)x100

It is 98.9%.

Example 2

Starch modification

One proceeds as for example 1 but one uses 0.2 eq of periodate during the oxidation reaction. One obtains a starch oxidised at 20%.

One proceeds in the same way as for example 1 for the coupling reaction and one obtains a starch oxidised at 20% and coupled to the DTCZ at 7%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The radiochemical purity (RCP) is 99%.

Example 3

Starch modification

One proceeds as for example 1 but one uses 0.1 eq of periodate during the oxidation reaction. One obtains a starch oxidised at 10%.

One proceeds in the same way as for example 1 for the coupling reaction and one obtains a starch oxidised at 10% and coupled to the DTCZ at 7%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The radiochemical purity (RCP) is 98.8%.

Example 4

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of N-methyl-S-methyl dithiocarbazate ($NH_2N(CH_3)(C=S)SCH_3$), M=136, that is 1.50 g dissolved in 10 ml of ethanol. The suspension is stirred for 18 hours at room temperature. The solution

is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 5%, which corresponds to a coupling level of the N-methyl S-methyl dithiocarbazate of 6.5% (6.5 units dithiocarbazate for 100 theoretical aldehyde functions, that is 13 dithiocarbazate units for 100 units glucose). The coupling yield is thus 65%.

Labelling reaction to ^{99m}Tc

One proceeds as for example 1. The RCP is 95%.

Example 5

Starch modification

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of 4-phenyl 3-thiosemicarbazide $(NH_2NH)(C=S)NH(C_6H_5)$, M=167, that is 1.83 g dissolved in 10 ml of ethanol. The suspension is stirred for 18 hours at room temperature. The solution is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 3.16%, which corresponds to a coupling level of the 4-phenyl 3-thiosemicarbazone of 8% (8 units thiosemicarbazide for 100 theoretical aldehyde functions, that is 16 thiosemicarbazone units for 100 units glucose). The coupling yield is thus 80%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 98%.

Example 6

Starch modification

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of 4-methyl 3-thiosemicarbazide $(NH_2NH)(C=S)NH(CH_3)$, M=105, that is 1.15 g dissolved in 10 ml of ethanol. The suspension is shaken for 18 hours at room temperature. The solution is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 2.9%, which corresponds to a coupling level of the 4-methyl 3-thiosemicarbazone of 7.3% (7.3 units thiosemicarbazide for 100 theoretical aldehyde functions, that is 14.6 thiosemicarbazone units for 100 units glucose). The coupling yield is thus 73%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 97%.

Example 7

Starch modification

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of 4,4-dimethyl 3-thiosemicarbazide $(NH_2NH)(C=S)N(CH_3)_2$, M=119, that is 1.30 g dissolved in 10 ml of ethanol. The suspension is

stirred for 18 hours at room temperature. The solution is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 3%, which corresponds to a coupling level of the 4,4-dimethyl 3-thiosemicarbazone of 7.5% (7.5 units thiosemicarbazide for 100 theoretical aldehyde functions, that is 15 thiosemicarbazone units for 100 units glucose). The coupling yield is thus 75%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 96%.

Example 8

Starch modification

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of 4-allyl 3-thiosemicarbazide $(NH_2NH)(C=S)NH(CH_2CH=CH_2)$, M=131, that is 1.44 g dissolved in 10 ml of ethanol. The suspension is stirred for 18 hours at room temperature. The solution is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 3%, which corresponds to a coupling level of the 4-allyl 3-thiosemicarbazone of 7.5% (7.5 units thiosemicarbazide for 100 theoretical aldehyde functions, that is 15 thiosemicarbazone units for 100 units glucose). The coupling yield is thus 75%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 98%.

Example 9

Starch modification

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of 3-thiosemicarbazide $(NH_2NH)(C=S)NH_2$, M=91, that is 1 g dissolved in 10 ml of ethanol. The suspension is stirred for 18 hours at room temperature. The solution is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 2.9%, which corresponds to a coupling level of the 3-thiosemicarbazone of 7.3% (7.3 units thiosemicarbazide for 100 theoretical aldehyde functions, that is 14.6 thiosemicarbazone units for 100 units glucose). The coupling yield is thus 73%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 95%.

Example 10

Starch modification

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of 2-aminothiophenol $(C_6H_4)(NH_2)(SH)$, M=125, that is 1.37 g dissolved in

10 ml of ethanol. The suspension is stirred for 18 hours at room temperature. The solution is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 3%, which corresponds to a coupling level of the 2-aminothiophenol of 7.5% (7.5 units aminothiophenol for 100 theoretical aldehyde functions, that is 15 aminothiophenol units for 100 units glucose). The coupling yield is thus 75%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 94%.

Example 11

Starch modification

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of 2-mercaptoethylamine (or 2-aminoethanethiol) ($NH_2CH_2CH_2SH$), M=91, that is 1 g dissolved in 10 ml of ethanol. The suspension is stirred for 18 hours at room temperature. Next one adds 0.015 mole of sodium borohydride ($NaBH_4$) so as to reduce the Schiff base formed to stabilise it (non-aromatic Schiff bases not being stable) and leaves it to react for 1 hour. The solution is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a

sulphur content of 3.4%, which corresponds to a coupling level of the 2-mercaptoethylamine of 8.5% (8.5 units 2-mercaptoethylamine for 100 theoretical aldehyde functions, that is 17 2-mercaptoethylamine units for 100 units glucose). The coupling yield is thus 85%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 95%.

Example 12

Starch modification

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of 2-amino 4-mercaptotriazole $(C_4N_2H)(SH)(NH_2)$, M=116, that is 1.27 g dissolved in 10 ml of ethanol. The suspension is stirred for 18 hours at room temperature. The solution is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 2.8%, which corresponds to a coupling level of the 2-amino 4-mercaptotriazole of 7% (7 units mercaptotriazole for 100 theoretical aldehyde functions, that is 14 mercaptotriazole units for 100 units glucose). The coupling yield is thus 75%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 85%.

Comparative example 1

One uses 10 g of sieved pharmacopoeia maize which has not undergone chemical transformation and one

caries out the same procedure as in example 1 to label it with ^{99m}Tc.

The RCP is 19%.

This example is a good demonstration of the fact that the chemical modification (fixation of sequestering groups) carried out according to the invention is certainly necessary to allow labelling with ^{99m}Tc. In addition, one cannot obtain durable pulmonary captation if the microparticles are labelled without prior chemical transformation contrary to the product relating to the present invention. These results are therefore in contradiction with that which is described in FR-A-2 273 516.

Example 13

Cellulose modification

One proceeds as in example 1 but using sieved cellulose between 10 and 40 µm. Thus one obtains 10 g of cellulose oxidised at 30% and coupled to the DTCZ at 7%.

Labelling reaction to ^{99m}Tc

One proceeds as in example 1. The RCP is 99.1%.

Example 14

Sprague Dawley rats weighing about 200 g are anaesthetised with sodium thiopental, and are injected intravenously with different solutions of microspheres labelled with ^{99m}Tc according to examples 1 to 9 and 13. Each animal receives 0.2 ml of solution in the penis vein, that is 0.2 mc per animal. The animals are then placed under a gamma-ray camera and successive static images are shot over a period of 3 hours. One thus obtains successive images after acquiring 15,000 shots

per image. Then, manually, one defines the zones of interest in order to estimate the activity present in the different organs 15 minutes after the injection. The results are given in tables I below.

Tables I: Results

% activity 15 min. after I.V.	Example 1	Example 2	Example 3	Example 4	Example 5
% pulmon. activity	90%	85%	80%	80%	85%
% hepat. activity	<5%	<5%	<5%	<10%	<10%
pulmon. half-life	2 hours	1 hour	30 mins	2 hours	2 hours

% activity 15 min. after I.V.	Example 6	Example 7	Example 8	Example 9	Example 10
% pulmon. activity	85%	85%	85%	85%	90%
% hepat. activity	<5%	<5%	<5%	<5%	<5%
pulmon. half-life	2 hours	2 hours	2 hours	2 hours	> 4 hours

One thus notes that the modified microspheres show very good pulmonary captation. In addition, one can modulate the speed of pulmonary elimination by varying

the oxidation level as shown in examples 1, 2 and 3 (oxidation levels 30, 20 and 10%).

The usage of cellulose makes it possible to lengthen the speed of elimination considerably (example 10, half-life > 4 hours).

Comparative example 2

In this example, natural starch is not used, but microspheres prepared from amyl pectin reticulated by epichlorhydrin as in the patent FR-A-2 273 516.

Preparation of reticulated microspheres of starch

One dissolves 8 g of maize amyl pectin in 40 ml of a solution containing 4 g of NaOH and 0.15 g of sodium borohydride. The amyl pectin is left for 24 hours to dissolve. Next one prepares an emulsion by stirring 60 ml of fluid paraffin and 1.6 g of soy lecithin dissolved in 4 ml of hexane at 800 revs/min. Then one adds the aqueous phase containing the amyl pectin and then 3.2 ml of epichlorhydrin. The emulsion is heated to 55°C for 4 hours and then left to be stirred overnight. The microspheres obtained of a size around 50 µm are washed by 3 times 250 ml of acetone, dried and then lyophilised.

Labelling with ^{99m}Tc

One proceeds as for example 1 but using 1 mg of $SnCl_2$, $2H_2O$. The RCP is 90%.

Starch modification

One proceeds as for example 1 but using 10 g of microspheres of amyl pectin reticulated by the epichlorhydrin previously prepared. One thus obtains 10 g of microspheres of amyl pectin oxidised at 30% and coupled to the DTCZ at 7%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 99%.

Example 15

One follows the same operational mode as in example 14 to test the microspheres of reticulated amyl pectin labelled with ^{99m}Tc of the comparative example 2. The results obtained are given in table II below.

Table II

% activity 15 min. after I.V.	Comparative example 2	Example 17
% pulmonary activity	< 10%	85%
% hepatic activity	70%	<5%
pulmonary half-life	-	2 hours

One notes that contrary to the description in FR-A-2 273 516 the microspheres of reticulated amyl pectin not modified chemically are labelled by ^{99m}Tc but do not present any pulmonary captation, doubtless due to the weak link between ^{99m}Tc and the microspheres. On the other hand, these microspheres transformed chemically by the procedure of the invention demonstrate good pulmonary captation.

Example 16

Starch microspheres prepared as in example 1 (starch oxidised at 30%, coupled with DTCZ at 7%) are used to produce sterile labelling kits and are ready for labelling with ^{99m}Tc.

Sterilisation of the microspheres

10 g of microspheres are introduced into a flask crimped and then irradiated by a source of cobalt-60.

The microspheres receive a total dose of gamma radiation of 25kGy over 20 hours.

Preparation of the kits

200 mg of sterilised microspheres are introduced in a sterile way into a reactor containing 20 ml of NaCl 9/1000. The solution is de-aerated by nitrogen bubbling and then one adds 400 µl of a sterile solution of $SnCl_2$, $2H_2O$ at 0.5 mg/ml in HCl 0.1N. One separates 1 ml of solution for each of 20 flasks, which are then lyophilised and placed in a nitrogen atmosphere.

Each flask thus contains:

10 mg of modified microspheres

10 µg of $SnCl_2$, $2H_2O$

9 mg of NaCl

Labelling reaction with ^{99m}Tc

5 ml of TcO_4^- (5mc) solution is added to a flask in lyophilised form and left to react for 15 minutes.

One proceeds as in example 1. The RCP is 98.7%.

Kit stability trial

Labelling kits prepared as above are stored at different temperatures and then the labelling reaction to ^{99m}Tc is tested so as to evaluate their stability. The results obtained are given in table III below:

Table III : RCP (%)

Storage temperature	6 months	12 months
2-8°C	98.5%	98.4%
25°C	97%	96%
45°C	94%	90%

One notes the very high stability of the kit stored at a temperature between 2 and 8°C.

Example 17

Sprague Dawley rats weighing about 200 g are anaesthetised with sodium thiopental, and are then injected intravenously with different solutions of microspheres labelled with ^{99m}Tc as in example 14. Each animal receives 0.2 ml of solution in the penis vein, that is 0.2 mc per animal. The animals are then killed 15 minutes after the injection. Next, the different organs are retrieved, the measurement of radioactivity present in each organ is counted and thus the percentage of activity present in each organ is determined. The results are given in table IV below:

Table IV - Results

% of the dose injected 15 minutes after injection

Organs	Example 1	Example 13
Blood (1 ml)	0.1%	0.2%
Liver	2.2%	5.6%
Kidneys	0.4%	0.4%
Lungs	91%	82%
Spleen	0.1%	0.1%
Intestines	1.5%	0.7%
Bladder	0.1%	1.3%

One thus notes a very high pulmonary captation whereas as there is weak captation in the other organs for natural starch microspheres as well as reticulated starch base microspheres. The sterile product prepared under kit form thus seems completely adapted to usage as a radiopharmaceutical product for pulmonary perfusion substituting for albumin, and for radiotherapy.

Exampl 18

One uses cellulose oxidised at 30% and coupled with the DTCZ at 7% as in example 13. The cellulose thus modified is labelled with rhenium 186 in order to illustrate the utilisation of the support according to the present invention for therapy.

Labelling reaction with Re 186

10 mg of modified cellulose are introduced into a penicillin type flask. One then adds 2 ml of physiological serum and then 20 mg of citric acid and finally 1 mg of $SnCl_2$, $2H_2O$ (100 µl of a solution at 10 mg/ml in HCl 0.1 N).

Next one adds to the contents of the flask 0.1 ml of ReO_4^- solution corresponding to an activity of 2 mc. The flask is heated in a water bath at 100°C for 30 minutes. The radiochemical purity (RCP) is determined by filtration on a Millipore as in example 1.

RCP = 92%

In order to prove the stability of the link between rhenium 186 and the cellulose microspheres, a stability test *in vitro* is carried out.

The mixture is incubated with HSA (human serum albumin) at 20 mg/ml at 37°C. The following results are obtained:

Incubation time	0	2 hours	6 hours	24 hours	48 hours
RCP	92%	92%	91%	89%	90%

These results therefore prove the very high stability of labelling of cellulose microspheres with rhenium 186 and the high stability of the microspheres themselves vis-à-vis HSA.

The expert will easily understand that these results can be extrapolated to the utilisation of rhenium 188.

Example 19

Sprague Dawley rats weighing about 200 g are anaesthetised and then injected with 0.2 ml (0.1 mc) of cellulose microsphere solution labelled with Re 186 as in example 18.

The animals are then placed under a gamma-ray camera and images are registered over 48 hours.

The activity present in the zones of interest is then calculated as in example 14.

Time after injection	1 hour	2 hours	6 hours	24 hours	48 hours
% pulmon. activity	80%	85%	85%	85%	90%
% hepatic activity	<5%	<5%	<5%	<5%	<5%

These results therefore prove that the activity remains blocked at the pulmonary level for at least 48 hours. This type of microsphere can thus be used for therapy.

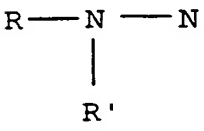
The most important clinical application can be the treatment of liver cancers after injection, not intravenously but directly into the hepatic artery (metabolic radiotherapy).

Another possible application is to inject this type of particle subcutaneously in breast cancer. The particles migrating through the lymphatic system may

make it possible to treat the sentinel nodes invaded by cancerous cells.

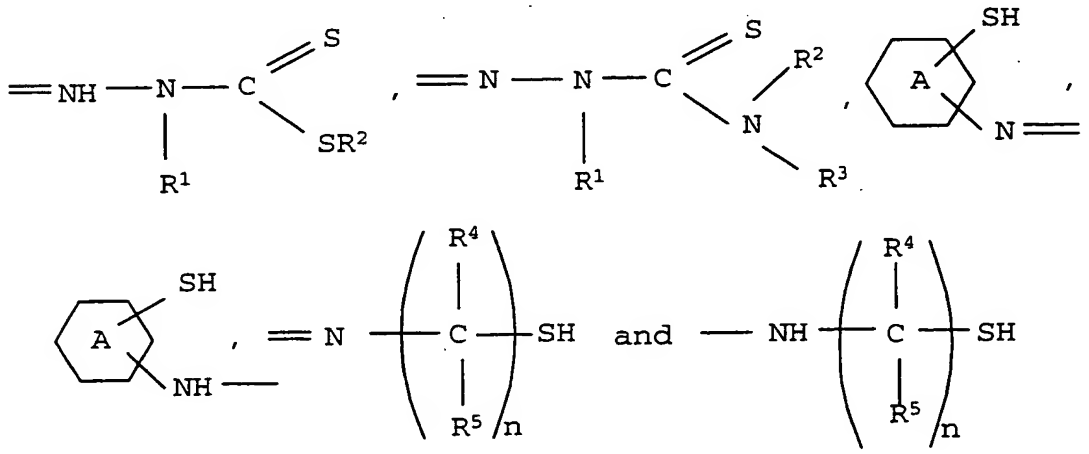
CLAIMS

1. A radiopharmaceutical product comprising a polysaccharide provided with sequestering groups linked to the polysaccharide by covalent bonds and chosen from among the groups of formulae R-NH-, R-N=, and



in which R is a hydrocarbonic or aromatic group comprising at least one atom of sulphur, and R' is an atom of hydrogen or an alkyl or methyl grouping, said sequestering groups forming, together with a radioactive metal chosen from among technetium, rhenium, copper, yttrium, erbium, gallium and samarium, a complex of the chelate type, in which the polysaccharide is in the form of microparticles.

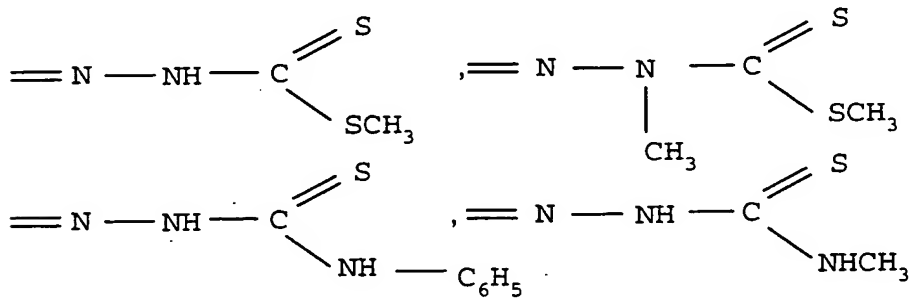
2. A radiopharmaceutical product according to Claim 1 in which the sequestering groups are chosen from among the groups of formulae:

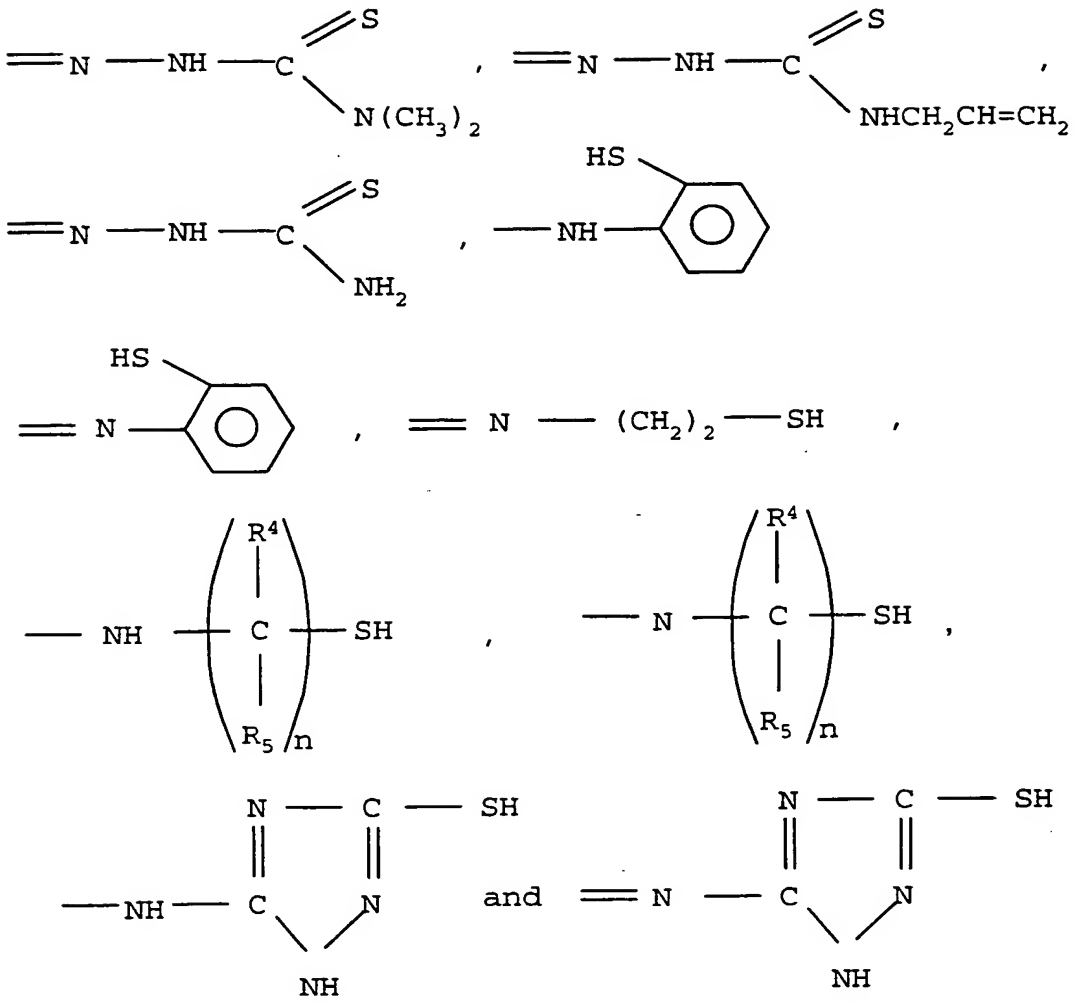


in which R^1, R^2, R^3, R^4 and R^5 are independently atoms of hydrogen atoms, saturated or unsaturated hydrocarbonic groups, carboxylic groups or aromatic groups,

is an aromatic nucleus possibly containing one or several heteroatoms, and n is a whole number between 1 and 5.

3. A radiopharmaceutical product according to Claim 2 in which the sequestering groups are chosen from among the groups of formulae:





4. A radiopharmaceutical product according to any one of Claims 1 to 3, in which the polysaccharide is chosen from among natural starch, cellulose and reticulated amyl pectin.

5. A radiopharmaceutical product according to any one of Claims 1 to 5, in which the microparticles have a dimension between 0.01 and 100 µm

6. A radiopharmaceutical product according to any one of Claims 1 to 5, in which the level of

sequestering groups is from 0.1 to 50% relative to the saccharide patterns of polysaccharide.

7. Utilisation of a radiopharmaceutical product according to any one of Claims 1 to 6, in which the radioactive metal is ^{99m}Tc or ^{67}Ga to prepare a product intended for diagnosis.

8. Utilisation of a radiopharmaceutical product according to any one of Claims 1 to 6, in which the radioactive metal is rhenium-186 or 188, copper-64 or 67, yttrium 90, erbium 169 or samarium 153, to prepare a drug.

9. Utilisation of a radiopharmaceutical product according to any one of Claims 1 to 7, in which the radioactive metal is ^{99m}Tc to prepare a product intended for pulmonary scintigraphy.

10. A radiopharmaceutical product according to any one of Claims 1 to 6, under the form of a suspension of microspheres in a physiologically acceptable liquid or in lyophilised form.

11. A procedure for preparation of a radiopharmaceutical product according to any one of Claims 1 to 6, which comprises the following stages:

(a) submit a polysaccharide to an oxidation carried out by means of a periodate,

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(71) Applicant *(for all designated States except US)*: **CIS BIO INTERNATONIAL** [FR/FR]; Route Nationale 306, F-91400 Saclay (FR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BELLANDE, Emmanuel** [FR/FR]; 85, avenue Paul Doumer, F-91160 Saulx les Chartreux (FR). **JALLET, Pierre** [FR/FR]; La Membrolle sur Longuenée, F-49770 Au Lion D'Angers (FR). **DENIZOT, Benoît** [FR/FR]; 79 Bld Eugène Chaumin, F-49000 Angers (FR).

(74) Agent: **DES TERMES, Monique**; Brevatome, 3, rue du Docteur Lancereaux, F-75008 Paris (FR).

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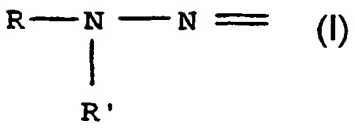


(I)

(57) Abstract: The present invention relates to radiopharmaceutical products and their preparation procedure. These products can be used for pulmonary scintigraphy or for therapy. They comprise a polysaccharide and sequestering groups of formulae R-NH-, R-N=, and formula (I) in which R is a hydrocarbonic or aromatic group comprising at least one atom of sulphur, and R' is an atom of hydrogen or an alkyl grouping such as methyl, said sequestering groups forming a chelate type complex with a radioactive metal such as technetium.

RADIOPHARMACEUTICAL PRODUCTS AND THEIR PREPARATION PROCEDURE

Technical field

The present invention relates to radiopharmaceutical products which can be used for diagnosis or therapy and to their preparation procedure.

In particular, it relates to radiopharmaceutical products formed for example from a suspension of particles labelled by a radioactive isotope utilisable in particular for pulmonary scintigraphy, for example in order to establish a diagnosis when a pulmonary embolism is suspected.

In this application, the products are used under the form of particles which are preferably spherical in shape and of a size ranging from 10 to 100µm. In fact, since the pulmonary capillaries have a diameter of about 7µm, the particles remain blocked in the capillaries after their intravenous injection, which makes it possible to visualise anomalies of pulmonary blood perfusion.

Evidently these products must fulfil a certain number of pharmaceutical restrictions. In particular they must have a suitable degradation rate *in vivo*, that is sufficiently slow to allow imagery to be carried out, for example by a gamma-ray camera, a minimum of about one hour, but also sufficiently rapid so as not to provoke permanent obstruction of the pulmonary capillaries, which could give rise to small thromboses. In addition, these products must not be

toxic for the organism, they must be able to be sterilised for example by autoclaving or by irradiation, they must be able to be labelled easily with a radioactive metal and be able to be packaged under the form of a stable labelling kit.

Prior Art

For example, the application for French brevet FR-A-2 273 516, deposited in 1975 by the PHARMACIA AKTIEBOLAG Company, resident in Sweden, describes the use of microspheres of amyl pectin reticulated by epichlorhydrin and labelled by a simple mixture with ^{99m}Tc for pulmonary perfusion scintigraphy. These particles present several inconveniences. In fact, only the hydroxyl groupings of amyl pectin used can allow this mixture labelling, and unfortunately they only form weak bonds with technetium and do not make stable labelling possible. In addition, the preparation procedure described uses many solvents and emulsifiers which are difficult to eliminate from the particles prepared. Furthermore, the exact rate of reticulation cannot be measured accurately nor controlled on this particle type.

Moreover, this document does not describe the kit compatible with routine utilisation in nuclear medicine. In fact, for an injectable preparation for humans, several manipulations such as adjunction of tin to the sterile flask, a centrifuging, a restoration of suspension, etc. are necessary, which is not compatible with sterility requirements.

Finally, the solutions obtained are not stable and the epichlorhydrin used for reticulation is recognised as being very toxic and mutagenic.

The inventors demonstrated other defects of these microparticles in the comparative examples 1 and 2 below.

The application for French brevet FR-A-2 285 857 deposited in 1975 by the PHARMAGIA FINE CHEMICALS AB Company, resident in Sweden, describes the utilisation of polysaccharide particles linked to different sequestering agents and labelled with the aid of radioactive isotopes. The particles comprise chelating groups linked by covalent bonds to which the radioactive nucleus is linked under the form of chelate type complexes which are principally composed of at least four, and preferably at least five to eight cyclic nuclei with 5 to 6 groups, enclosing the metal, and two metal-coordinating atoms. The polysaccharide is a polysaccharide reticulated chemically, for example by means of epichlorhydrin or epibromhydrin. Leaving the labelling aside, these particles present the same problems as those mentioned previously for the particles described in FR-A-2 273 516. Moreover, this document does not give any examples of labelling with technetium. Further, the labelling procedure comprises heating to 100°C in the presence of the radioactive element, a washing and a drying after labelling, which is not at all compatible with the idea of the above-mentioned labelling kit and the restrictions of sterility of usage.

Even though the labelling method described allows the particles to be labelled in a relatively stable manner, it does not make it possible to prepare a labelling kit which is pharmaceutically acceptable, in particular because it contains epichlorhydrin, and easily usable in a nuclear medicine service.

The microspheres described in these two brevet applications are thus not adapted to the pharmaceutical restrictions and they cannot be exploited. Moreover they have never been used for pulmonary scintigraphy. This type of product has been abandoned since.

The many researches carried out since 1975 for perfecting new radiopharmaceutical products have concentrated on products based on albumin-serum and its derivatives. These blood products do in fact correspond to pharmaceutical restrictions and can be used in particular for pulmonary scintigraphy. These are the products used at present in nuclear medicine.

For example, in 1975, M.A. Davis, in the document "Radiopharmaceuticals N.Y.", 1975, pages 267 to 281, described the radioactive particles intended for the study of pulmonary perfusion. The particles described in this document are macro-aggregates of radio-iodinated serum albumin (^{131}I-MAA) or microspheres of denatured human serum albumin labelled with technetium (^{99m}Tc-HAM). The microspheres of ^{99m}Tc-HAM are preferable, because of their uniformity of particle size ranging essentially between 40 and 50 µm. Moreover this document describes the general characteristics required for such radiopharmaceutical particles.

The document of R. Guiraud "Macro-aggregates and radioactive microspheres", Radiopharmaceuticals, 1997, 519, describes macro-aggregates of albumin (MAA) and microspheres of human serum albumin. It describes the labelling of such micro-aggregates and microparticles with technetium 99m by a solution of stannous chloride. It also notes that the optimum size for the microparticles is 15±5 µm. It mentions organic microspheres of starch.

At present, these macro-aggregates and microspheres of human serum albumin labelled with ^{99m}Tc are by far the most utilised in nuclear medicine. However, they present several inconveniences. For example, the variability and quality of batches of human albumin sometimes make preparation of diagnosis kits difficult, containing particles which can vary in size and number. But one of the major inconveniences is their human origin, which can pose serious problems of potential vital contamination of the type HIV, hepatitis, or Creutzfeld-Jacob disease.

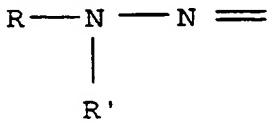
It would therefore be very interesting to be able to have microspheres labelled with ^{99m}Tc which are not of human origin in order to ensure perfect safety.

With this in view, the very recent document of A.C. Perkins, Nuclear Medicine Communications, 1999, 20, 1-3 describes ways of replacing radiopharmaceutical products obtained from blood. In particular it mentions the utilisation of recombinant materials, synthetic polymers and polypeptides. But, this document does not mention polysaccharides.

Description of the invention

The precise aim of the invention is to overcome the inconveniences mentioned above for prior art products, by providing a radiopharmaceutical product being able to be easily labelled, for example with ^{99m}Tc, presenting a very good pulmonary captation which has been demonstrated by inventors for rats, non-toxic, easily biodegradable, easily sterilisable and able to be packaged as a kit ready for labelling, stable and fulfilling the pharmaceutical restrictions for this type of product. These advantages and others will be evident from the following description.

The radiopharmaceutical product of the present invention is characterised in that it comprises a polysaccharide provided with sequestering agents linked to the polysaccharide by covalent bonds and chosen among the groups of formulae R-NH-, R-N=, and



in which R is a hydrocarbonic or aromatic group comprising at least one atom of sulphur, and R' is a hydrogen atom or an alkyl grouping, for example methyl, said sequestering groups forming a chelate type complex with a radioactive metal chosen from among technetium, rhenium, copper, yttrium, erbium, gallium and samarium.

The utilisable alkyl groups for R' can be linear or branched, and preferably they have 1 to 5 carbon atoms.

According to the invention, the polysaccharide can be soluble, or in the form of microparticles. According

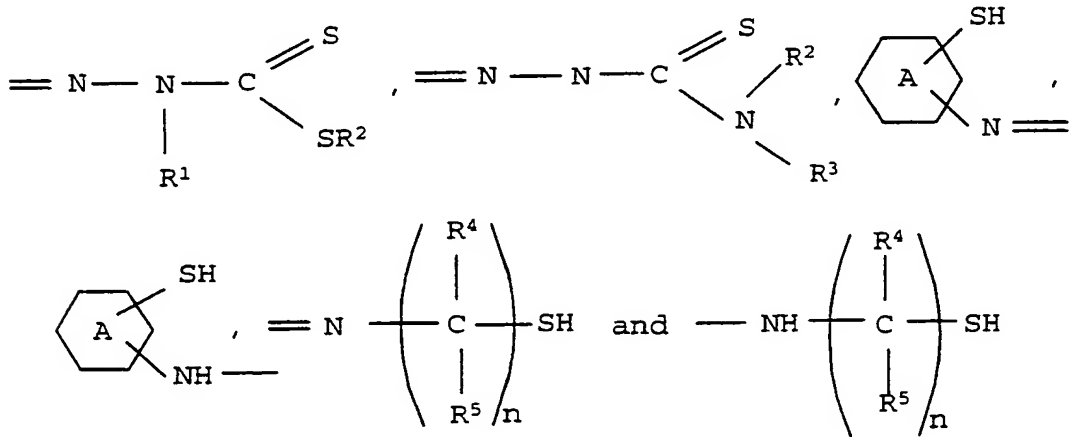
to the invention, the polysaccharide can be chosen, for example, from among natural starch, cellulose or reticulated amyl pectin.

The natural starch can, for example, be maize starch.

The polysaccharide can be in the form of microparticles, for example in the form of microspheres.

The present inventors have also demonstrated that modified cellulose according to the present invention offers very good pulmonary captation and an elimination speed slower than with starch. The modified cellulose of the present invention can therefore also be used for radiotherapy, for example with labelling with rhenium, copper, or with one of the above-mentioned metals, since it corresponds to the radiotherapy necessity of using microparticles with a longer half-life.

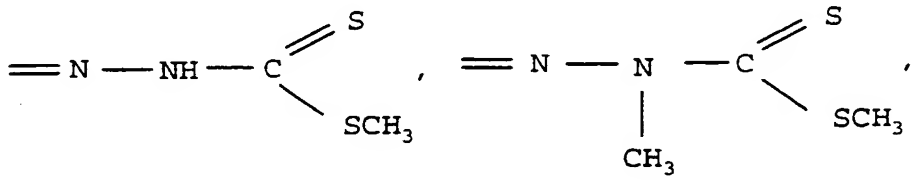
According to the invention, the sequestering groups can be chosen for example from the groups with formulae:

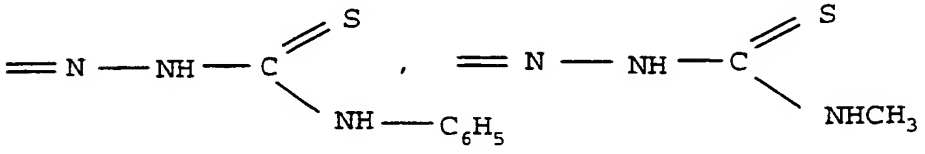


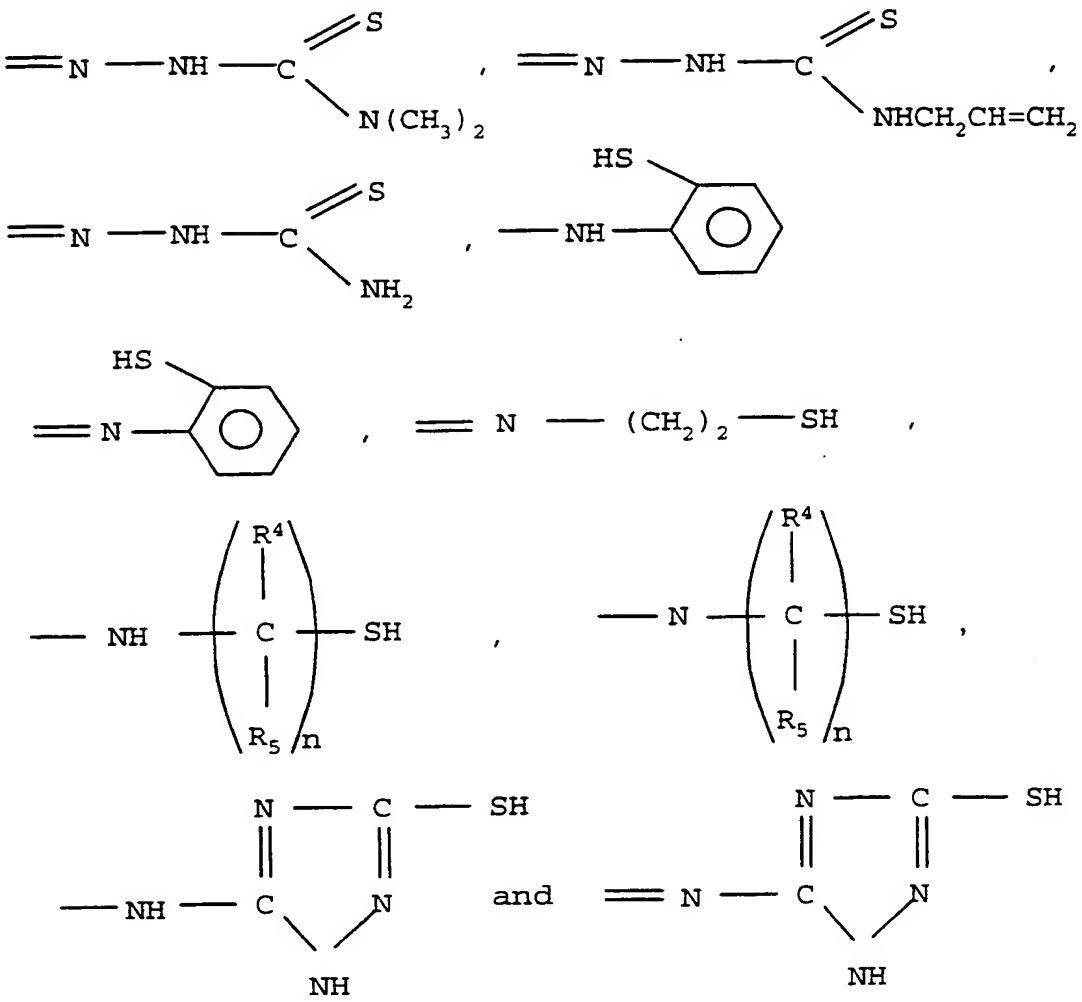
in which R^1, R^2, R^3, R^4 and R^5 are independently hydrogen atoms, saturated or unsaturated hydrocarbonic groups, carboxylic groups or aromatic groups,

is an aromatic nucleus possibly containing one or several hetero-atoms, and n is a whole number between 1 and 5.

For example, they can be chosen from among the formula groups:







According to the invention, the microparticles, for example in the shape of microspheres, can be of a dimension from 0.01 to 100 µm, preferably from 10 to 50 µm for diagnosis by pulmonary scintigraphy and from 0.1 to 5 µm for therapy.

According to the invention, the levels of sequestering groups can be from 0.1 to 50% compared to the saccharide patterns of the polysaccharide, preferably from 2 to 15%.

According to the invention, in the radiopharmaceutical product, particularly when it is

used for diagnosis, the radioactive metal can be ^{99m}Tc or gallium-67.

This can be the case, for example, when the radiopharmaceutical product is used for pulmonary scintigraphy.

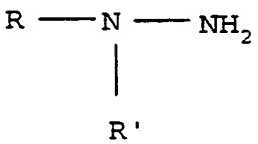
According to the invention, in the radiopharmaceutical product, in particular when it is used for therapy, the radioactive metal can be rhenium-186 or 188, copper-64 or 67, yttrium-90, erbium-169 or samarium-153.

According to the invention, said radiopharmaceutical product can be in the form of a suspension of microspheres in a physiologically acceptable liquid or in lyophilised form.

The present invention also provides a preparation procedure for the radiopharmaceutical product of the invention comprising the following stages:

(a) submit a polysaccharide, for example such as those mentioned above, to oxidation controlled by means of a periodate,

(b) react the oxidised polysaccharide with a compound containing a primary amine function or hydrazin of formula $R\text{-}NH_2$ or



(c) in which R is a hydrocarbonic or aromatic group comprising at least one atom of sulphur, in order to bond in a covalent manner to the polysaccharide with sequestering groups the metals of formulae R-NH-, R-

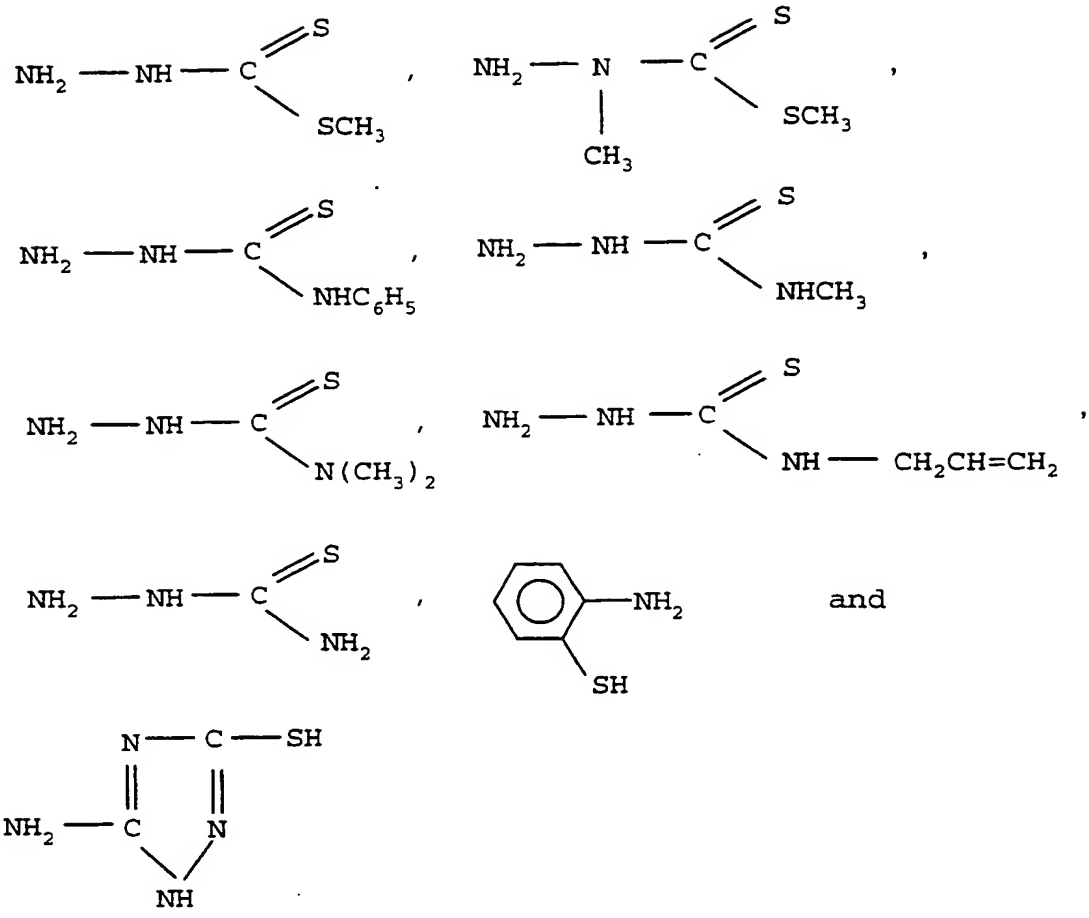
N= or R-NH-N=, and R' is a hydrogen atom or an alkyl grouping, for example methyl,

(d) react the polysaccharide comprising the sequestering groups with a salt of a radioactive metal chosen from among technetium, rhenium, copper, yttrium, erbium and samarium.

The oxidation controlled by means of a periodate can be that described, for example, in C.L. Mehltretter, "Methods in Carbohydrate Chemistry", vol. IV, 1964, applied in particular to the oxidation of starch, dextrane or cellulose. It is used in the examples given below.

According to the invention, the compound containing a primary amine function can correspond to the formula $NH_2-(CH_2)_n-SH$ with n being a whole number between 1 and 5, and can include a supplementary reducing stage of this compound with sodium borohydride between stages (b) and (c).

According to the invention, the compound bonded to the polysaccharide can correspond, for example, to one of the following formulae:

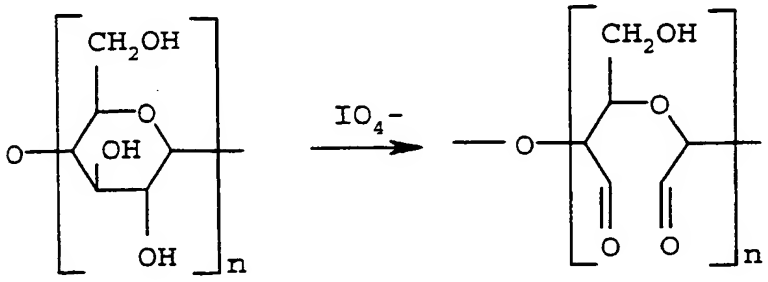


According to the invention, the level of sequestering groups fixed on the polysaccharide can be regulated by controlling the level of oxidation of the polysaccharide in stage (a) mentioned above. This oxidation level of polysaccharide can, for example, be between 10 to 50%. The level of the sequestering group can, for example, be between 2 and 15%.

In order to allow labelling of the polysaccharide according to the invention, for example with ^{99m}Tc, the inventors have therefore used a two-stage transformation method.

This method can be presented as consisting of carrying out a controlled oxidation of the

polysaccharide by the periodate in the first stage. Each unit of oxidised glucose thus generates two aldehyde groups in neighbouring positions following the chemical reaction diagram given below:

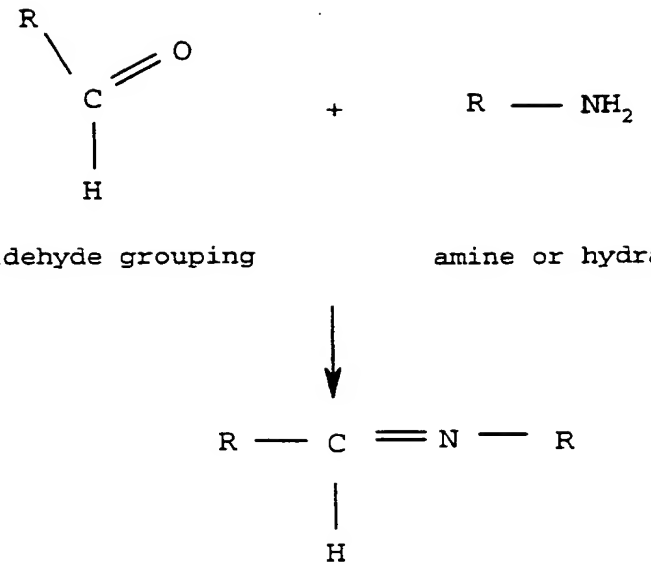


natural glucoside monomer oxidised glucoside monomer

The level of oxidation of the polysaccharide can be variable and easily adjusted. In fact, the yield of this oxidation reaction is close to 100% and the level of oxidaton can be calculated from the quantities of periodate added. In general, oxidation levels lower than 50% are used so as to modify the structure of the macromolecule only slightly. The real oxidation level, ranging from 1 to 100%, can easily be determined by a colorometric method.

In the second stage, the oxidised polysaccharide is made to react with a molecule containing an amine or hydrazin grouping with the general formula RNH_2 or $RNHNH_2$ to form a chelating grouping able to sequester technetium. Thus one obtains a Schiff base type ligand or thiosemicarbazone.

This second stage can be summarised as follows:



with:

1. $R=NR_1(C=S)SR_2$ (Schiff bases issued from dithiocarbazate
2. $R=NR_1(C=S)NR_2R_3$ (thiosemicarbazones)
3. R= aromatic grouping (aromatic Schiff bases)
4. R= alkyl grouping (alkylic Schiff bases); in this case the Schiff bases are not stable and one carries out a second reduction stage of the C=N bond with borohydride so as to stabilise it and then an amine C-NHR bond is obtained.

According to the invention, stage (c) can for example consist of putting into contact the microspheres of polysaccharide comprising the sequestering groups for example with a solution of pertechnetate $^{99m}TcO_4^-$ in the presence of a reducing agent, for example stannous chloride.

According to the invention, microparticles, for example microspheres, for example maize starch or

starch with a base of reticulated amyl pectin can thus be oxidised, then coupled to a molecule containing an amine or hydrazin function, for example S-methyl dithiocarbazate. These particles modified in this way can easily be labelled with, for example, ^{99m}Tc.

The present invention thus provides in particular microparticles prepared for example from a base of starch particles, which therefore do not present the inconveniences of the albumin mentioned above. In addition, the starch is described as an excipient in the pharmacopoeia. It is therefore easily available and at low cost.

The microparticles of the present invention also have the advantage of being able to be sterilised easily, for example by irradiation, and to be processed under the form of a kit ready for labelling.

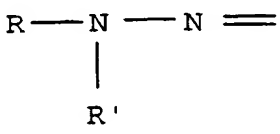
Moreover, the present inventors have demonstrated according to the present invention that the speed of pulmonary clearance can be modified according to the level of oxidation of the microparticles used in the present invention, which is not possible, for example, with human albumin microspheres.

Another advantage of the present invention lies in the simplicity of operation of the procedure: the reaction conditions being very gentle: reactions at ambient temperature, in an aqueous medium, quasi-quantitative yields. In addition, the sequestering reactions, for example with technetium, are quantitative; they take place at room temperature and without final purification which makes it possible to adapt to the requirements of sterility and simplicity

of preparation necessary for the utilisation of technetised kits in the hospital environment.

The present invention also provides a diagnosis kit which can be used, for example, for pulmonary scintigraphy. This kit comprises:

a first flask containing a polysaccharide according to the invention, that is to say provided with sequestering groups linked to the polysaccharide by covalent bonds and chosen among the groups of formulae R-NH-, R-N= and



in which R is a hydrocarbonic or aromatic group comprising at least one atom of sulphur, and in which R' is a hydrogen atom or an alkyl grouping such as methyl.

According to the invention, the polysaccharide can, for example, be in the form of microparticles, for example in the shape of microspheres, the microparticles being able to be in lyophilised form or in suspension in a pharmaceutically acceptable liquid.

The kit of the present invention can furthermore comprise a second flask containing stannous chloride preferably uin lyophilised form, or also when the polysaccharide is in lyophilised form, for example in the form of microparticles, in the first flask, said first flask can besides contain lyophilised stannous chloride.

The kits of the present invention are stable for at least twelve months as demonstrated in the examples given below.

The radiopharmaceutical product of the present invention therefore presents all the qualities required for a use such as radiopharmaceutical usage, for example for scintigraphy of pulmonary perfusion or for radiotherapy.

Other advantages will also appear when reading the following examples related to the present invention.

EXAMPLES

Example 1

A suspension of 10 g of maize starch from the pharmacopoeia is prepared, previously sieved between 10 and 40 µm, containing about 10% water, that is 0.055 mole of glucose in 100 ml of water. One adds 0.0168 mole of sodium periodate (0.3 eq) ($NaIO_4$), that is 3.6 g, dissolved in 100 ml of water. The suspension is then stirred for 18 hours at room temperature. The solution is filtered and the oxidised starch is rinsed by 5 times 20 ml of water and then 2 times 50 ml of acetone. The starch is vacuum dried and one obtains 10 g of starch oxidised at 30% (yield = 100%).

A suspension of 10 g of starch oxidised at 30% is prepared in 60 ml of a water/ethanol mixture 2/1 by volume. Next one adds 0.1 eq (0.1x0.055x2) that is 0.011 mole of S-methyl dithiocarbazate ($NH_2NH(C=S)SCH_3$), M=122, that is 1.34 g, dissolved in 10 ml of ethanol. The suspension is stirred for 18 hours at room temperature. The solution is next filtered and the

modified starch is washed by 3 times 20 ml of ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 5.4%, which corresponds to a coupling level of S-methyl dithiocarbazate (DTCZ) of 7% (7 units dithiocarbazate for 100 theoretical aldehyde functions that is 14 dithiocarbazate units for 100 glucose units). The coupling yield is therefore 70%. One thus obtains 10 g of starch oxidised at 30% and coupled to the DTCZ at 7%.

Labelling reaction with ^{99m}Tc

10 mg of starch thus modified are introduced into a flask of the penicillin type. One then adds 4 ml of physiological serum then 10 µg of $SnCL_2$, $2H_2O$ (20 µl of a solution at 0.5 mg/ml in HCl 0.1 N). Then 1 ml of a solution of $^{99m}TcO_4^-$ (5mc) is added. The solution is stirred for 15 minutes and the radiochemical purity control (RCP) is carried out by filtration of 1 ml of the solution on a Millipore filter of 0.22 µm then the filter is rinsed by 2ml of physiological serum. The labelled microspheres are retained on the filter contrary to the radioactive impurities not linked to the microspheres which are found in the filtrate. The radiochemical purity corresponds to:

RCP= (activity on the filter/total activity)x100

It is 98.9%.

Example 2

Starch modification

One proceeds as for example 1 but one uses 0.2 eq of periodate during the oxidation reaction. One obtains a starch oxidised at 20%.

One proceeds in the same way as for example 1 for the coupling reaction and one obtains a starch oxidised at 20% and coupled to the DTCZ at 7%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The radiochemical purity (RCP) is 99%.

Example 3

Starch modification

One proceeds as for example 1 but one uses 0.1 eq of periodate during the oxidation reaction. One obtains a starch oxidised at 10%.

One proceeds in the same way as for example 1 for the coupling reaction and one obtains a starch oxidised at 10% and coupled to the DTCZ at 7%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The radiochemical purity (RCP) is 98.8%.

Example 4

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of N-methyl-S-methyl dithiocarbazate ($NH_2N(CH_3)(C=S)SCH_3$), M=136, that is 1.50 g dissolved in 10 ml of ethanol. The suspension is stirred for 18 hours at room temperature. The solution

is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 5%, which corresponds to a coupling level of the N-methyl S-methyl dithiocarbazate of 6.5% (6.5 units dithiocarbazate for 100 theoretical aldehyde functions, that is 13 dithiocarbazate units for 100 units glucose). The coupling yield is thus 65%.

Labelling reaction to ^{99m}Tc

One proceeds as for example 1. The RCP is 95%.

Example 5

Starch modification

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of 4-phenyl 3-thiosemicarbazide $(NH_2NH)(C=S)NH(C_6H_5)$, M=167, that is 1.83 g dissolved in 10 ml of ethanol. The suspension is stirred for 18 hours at room temperature. The solution is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 3.16%, which corresponds to a coupling level of the 4-phenyl 3-thiosemicarbazone of 8% (8 units thiosemicarbazide for 100 theoretical aldehyde functions, that is 16 thiosemicarbazone units for 100 units glucose). The coupling yield is thus 80%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 98%.

Example 6

Starch modification

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of 4-methyl 3-thiosemicarbazide $(NH_2NH)(C=S)NH(CH_3)$, M=105, that is 1.15 g dissolved in 10 ml of ethanol. The suspension is shaken for 18 hours at room temperature. The solution is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 2.9%, which corresponds to a coupling level of the 4-methyl 3-thiosemicarbazone of 7.3% (7.3 units thiosemicarbazide for 100 theoretical aldehyde functions, that is 14.6 thiosemicarbazone units for 100 units glucose). The coupling yield is thus 73%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 97%.

Example 7

Starch modification

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of 4,4-dimethyl 3-thiosemicarbazide $(NH_2NH)(C=S)N(CH_3)_2$, M=119, that is 1.30 g dissolved in 10 ml of ethanol. The suspension is

stirred for 18 hours at room temperature. The solution is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 3%, which corresponds to a coupling level of the 4,4-dimethyl 3-thiosemicarbazone of 7.5% (7.5 units thiosemicarbazide for 100 theoretical aldehyde functions, that is 15 thiosemicarbazone units for 100 units glucose). The coupling yield is thus 75%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 96%.

Example 8

Starch modification

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of 4-allyl 3-thiosemicarbazide $(NH_2NH)(C=S)NH(CH_2CH=CH_2)$, M=131, that is 1.44 g dissolved in 10 ml of ethanol. The suspension is stirred for 18 hours at room temperature. The solution is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 3%, which corresponds to a coupling level of the 4-allyl 3-thiosemicarbazone of 7.5% (7.5 units thiosemicarbazide for 100 theoretical aldehyde functions, that is 15 thiosemicarbazone units for 100 units glucose). The coupling yield is thus 75%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 98%.

Example 9

Starch modification

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of 3-thiosemicarbazide $(NH_2NH)(C=S)NH_2$, M=91, that is 1 g dissolved in 10 ml of ethanol. The suspension is stirred for 18 hours at room temperature. The solution is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 2.9%, which corresponds to a coupling level of the 3-thiosemicarbazone of 7.3% (7.3 units thiosemicarbazide for 100 theoretical aldehyde functions, that is 14.6 thiosemicarbazone units for 100 units glucose). The coupling yield is thus 73%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 95%.

Example 10

Starch modification

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of 2-aminothiophenol $(C_6H_4)(NH_2)(SH)$, M=125, that is 1.37 g dissolved in

10 ml of ethanol. The suspension is stirred for 18 hours at room temperature. The solution is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 3%, which corresponds to a coupling level of the 2-aminothiophenol of 7.5% (7.5 units aminothiophenol for 100 theoretical aldehyde functions, that is 15 aminothiophenol units for 100 units glucose). The coupling yield is thus 75%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 94%.

Example 11

Starch modification

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of 2-mercaptoethylamine (or 2-aminoethanethiol) ($NH_2CH_2CH_2SH$), M=91, that is 1 g dissolved in 10 ml of ethanol. The suspension is stirred for 18 hours at room temperature. Next one adds 0.015 mole of sodium borohydride ($NaBH_4$) so as to reduce the Schiff base formed to stabilise it (non-aromatic Schiff bases not being stable) and leaves it to react for 1 hour. The solution is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a

sulphur content of 3.4%, which corresponds to a coupling level of the 2-mercaptoethylamine of 8.5% (8.5 units 2-mercaptoethylamine for 100 theoretical aldehyde functions, that is 17 2-mercaptoethylamine units for 100 units glucose). The coupling yield is thus 85%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 95%.

Example 12

Starch modification

One proceeds as for example 1 to obtain 10 g of starch oxidised at 30%. A suspension is prepared of 10 g of starch oxidised at 30% in 60 ml of a mixture of water/ethanol (2/1 by volume). Next one adds 0.1 eq (0.1x0.055x2), that is 0.011 mole of 2-amino 4-mercaptotriazole $(C_4N_2H)(SH)(NH_2)$, M=116, that is 1.27 g dissolved in 10 ml of ethanol. The suspension is stirred for 18 hours at room temperature. The solution is then filtered and the modified starch washed by 3 times 20 ml ethanol and then vacuum dried. One thus obtains about 10 g of modified starch. The assay of the powder by elementary analysis gives a sulphur content of 2.8%, which corresponds to a coupling level of the 2-amino 4-mercaptotriazole of 7% (7 units mercaptotriazole for 100 theoretical aldehyde functions, that is 14 mercaptotriazole units for 100 units glucose). The coupling yield is thus 75%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 85%.

Comparative example 1

One uses 10 g of sieved pharmacopoeia maize which has not undergone chemical transformation and one

caries out the same procedure as in example 1 to label it with ^{99m}Tc.

The RCP is 19%.

This example is a good demonstration of the fact that the chemical modification (fixation of sequestering groups) carried out according to the invention is certainly necessary to allow labelling with ^{99m}Tc. In addition, one cannot obtain durable pulmonary captation if the microparticles are labelled without prior chemical transformation contrary to the product relating to the present invention. These results are therefore in contradiction with that which is described in FR-A-2 273 516.

Example 13

Cellulose modification

One proceeds as in example 1 but using sieved cellulose between 10 and 40 µm. Thus one obtains 10 g of cellulose oxidised at 30% and coupled to the DTCZ at 7%.

Labelling reaction to ^{99m}Tc

One proceeds as in example 1. The RCP is 99.1%.

Example 14

Sprague Dawley rats weighing about 200 g are anaesthetised with sodium thiopental, and are injected intravenously with different solutions of microspheres labelled with ^{99m}Tc according to examples 1 to 9 and 13. Each animal receives 0.2 ml of solution in the penis vein, that is 0.2 mc per animal. The animals are then placed under a gamma-ray camera and successive static images are shot over a period of 3 hours. One thus obtains successive images after acquiring 15,000 shots

per image. Then, manually, one defines the zones of interest in order to estimate the activity present in the different organs 15 minutes after the injection. The results are given in tables I below.

Tables I: Results

% activity 15 min. after I.V.	Example 1	Example 2	Example 3	Example 4	Example 5
% pulmon. activity	90%	85%	80%	80%	85%
% hepat. activity	<5%	<5%	<5%	<10%	<10%
pulmon. half-life	2 hours	1 hour	30 mins	2 hours	2 hours

% activity 15 min. after I.V.	Example 6	Example 7	Example 8	Example 9	Example 10
% pulmon. activity	85%	85%	85%	85%	90%
% hepat. activity	<5%	<5%	<5%	<5%	<5%
pulmon. half-life	2 hours	2 hours	2 hours	2 hours	> 4 hours

One thus notes that the modified microspheres show very good pulmonary captation. In addition, one can modulate the speed of pulmonary elimination by varying

the oxidation level as shown in examples 1, 2 and 3 (oxidation levels 30, 20 and 10%).

The usage of cellulose makes it possible to lengthen the speed of elimination considerably (example 10, half-life > 4 hours).

Comparative example 2

In this example, natural starch is not used, but microspheres prepared from amyl pectin reticulated by epichlorhydrin as in the patent FR-A-2 273 516.

Preparation of reticulated microspheres of starch

One dissolves 8 g of maize amyl pectin in 40 ml of a solution containing 4 g of NaOH and 0.15 g of sodium borohydride. The amyl pectin is left for 24 hours to dissolve. Next one prepares an emulsion by stirring 60 ml of fluid paraffin and 1.6 g of soy lecithin dissolved in 4 ml of hexane at 800 revs/min. Then one adds the aqueous phase containing the amyl pectin and then 3.2 ml of epichlorhydrin. The emulsion is heated to 55°C for 4 hours and then left to be stirred overnight. The microspheres obtained of a size around 50 µm are washed by 3 times 250 ml of acetone, dried and then lyophilised.

Labelling with ^{99m}Tc

One proceeds as for example 1 but using 1 mg of $SnCl_2$, $2H_2O$. The RCP is 90%.

Starch modification

One proceeds as for example 1 but using 10 g of microspheres of amyl pectin reticulated by the epichlorhydrin previously prepared. One thus obtains 10 g of microspheres of amyl pectin oxidised at 30% and coupled to the DTCZ at 7%.

Labelling reaction with ^{99m}Tc

One proceeds as for example 1. The RCP is 99%.

Example 15

One follows the same operational mode as in example 14 to test the microspheres of reticulated amyl pectin labelled with ^{99m}Tc of the comparative example 2. The results obtained are given in table II below.

Table II

% activity 15 min. after I.V.	Comparative example 2	Example 17
% pulmonary activity	< 10%	85%
% hepatic activity	70%	<5%
pulmonary half-life	-	2 hours

One notes that contrary to the description in FR-A-2 273 516 the microspheres of reticulated amyl pectin not modified chemically are labelled by ^{99m}Tc but do not present any pulmonary captation, doubtless due to the weak link between ^{99m}Tc and the microspheres. On the other hand, these microspheres transformed chemically by the procedure of the invention demonstrate good pulmonary captation.

Example 16

Starch microspheres prepared as in example 1 (starch oxidised at 30%, coupled with DTCZ at 7%) are used to produce sterile labelling kits and are ready for labelling with ^{99m}Tc.

Sterilisation of the microspheres

10 g of microspheres are introduced into a flask crimped and then irradiated by a source of cobalt-60.

The microspheres receive a total dose of gamma radiation of 25kGy over 20 hours.

Preparation of the kits

200 mg of sterilised microspheres are introduced in a sterile way into a reactor containing 20 ml of NaCl 9/1000. The solution is de-aerated by nitrogen bubbling and then one adds 400 µl of a sterile solution of $SnCl_2$, $2H_2O$ at 0.5 mg/ml in HCl 0.1N. One separates 1 ml of solution for each of 20 flasks, which are then lyophilised and placed in a nitrogen atmosphere.

Each flask thus contains:

10 mg of modified microspheres

10 µg of $SnCl_2$, $2H_2O$

9 mg of NaCl

Labelling reaction with ^{99m}Tc

5 ml of TcO_4^- (5mc) solution is added to a flask in lyophilised form and left to react for 15 minutes.

One proceeds as in example 1. The RCP is 98.7%.

Kit stability trial

Labelling kits prepared as above are stored at different temperatures and then the labelling reaction to ^{99m}Tc is tested so as to evaluate their stability. The results obtained are given in table III below:

Table III : RCP (%)

Storage temperature	6 months	12 months
2-8°C	98.5%	98.4%
25°C	97%	96%
45°C	94%	90%

One notes the very high stability of the kit stored at a temperature between 2 and 8°C.

Example 17

Sprague Dawley rats weighing about 200 g are anaesthetised with sodium thiopental, and are then injected intravenously with different solutions of microspheres labelled with ^{99m}Tc as in example 14. Each animal receives 0.2 ml of solution in the penis vein, that is 0.2 mc per animal. The animals are then killed 15 minutes after the injection. Next, the different organs are retrieved, the measurement of radioactivity present in each organ is counted and thus the percentage of activity present in each organ is determined. The results are given in table IV below:

Table IV - Results

% of the dose injected 15 minutes after injection

Organs	Example 1	Example 13
Blood (1 ml)	0.1%	0.2%
Liver	2.2%	5.6%
Kidneys	0.4%	0.4%
Lungs	91%	82%
Spleen	0.1%	0.1%
Intestines	1.5%	0.7%
Bladder	0.1%	1.3%

One thus notes a very high pulmonary captation whereas as there is weak captation in the other organs for natural starch microspheres as well as reticulated starch base microspheres. The sterile product prepared under kit form thus seems completely adapted to usage as a radiopharmaceutical product for pulmonary perfusion substituting for albumin, and for radiotherapy.

Example 18

One uses cellulose oxidised at 30% and coupled with the DTCZ at 7% as in example 13. The cellulose thus modified is labelled with rhenium 186 in order to illustrate the utilisation of the support according to the present invention for therapy.

Labelling reaction with Re 186

10 mg of modified cellulose are introduced into a penicillin type flask. One then adds 2 ml of physiological serum and then 20 mg of citric acid and finally 1 mg of $SnCl_2$, $2H_2O$ (100 µl of a solution at 10 mg/ml in HCl 0.1 N).

Next one adds to the contents of the flask 0.1 ml of ReO_4^- solution corresponding to an activity of 2 mc. The flask is heated in a water bath at 100°C for 30 minutes. The radiochemical purity (RCP) is determined by filtration on a Millipore as in example 1.

RCP = 92%

In order to prove the stability of the link between rhenium 186 and the cellulose microspheres, a stability test *in vitro* is carried out.

The mixture is incubated with HSA (human serum albumin) at 20 mg/ml at 37°C. The following results are obtained:

Incubation time	0	2 hours	6 hours	24 hours	48 hours
RCP	92%	92%	91%	89%	90%

These results therefore prove the very high stability of labelling of cellulose microspheres with rhenium 186 and the high stability of the microspheres themselves vis-à-vis HSA.

The expert will easily understand that these results can be extrapolated to the utilisation of rhenium 188.

Example 19

Sprague Dawley rats weighing about 200 g are anaesthetised and then injected with 0.2 ml (0.1 mc) of cellulose microsphere solution labelled with Re 186 as in example 18.

The animals are then placed under a gamma-ray camera and images are registered over 48 hours.

The activity present in the zones of interest is then calculated as in example 14.

Time after injection	1 hour	2 hours	6 hours	24 hours	48 hours
% pulmon. activity	80%	85%	85%	85%	90%
% hepatic activity	<5%	<5%	<5%	<5%	<5%

These results therefore prove that the activity remains blocked at the pulmonary level for at least 48 hours. This type of microsphere can thus be used for therapy.

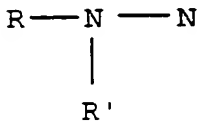
The most important clinical application can be the treatment of liver cancers after injection, not intravenously but directly into the hepatic artery (metabolic radiotherapy).

Another possible application is to inject this type of particle subcutaneously in breast cancer. The particles migrating through the lymphatic system may

make it possible to treat the sentinel nodes invaded by cancerous cells.

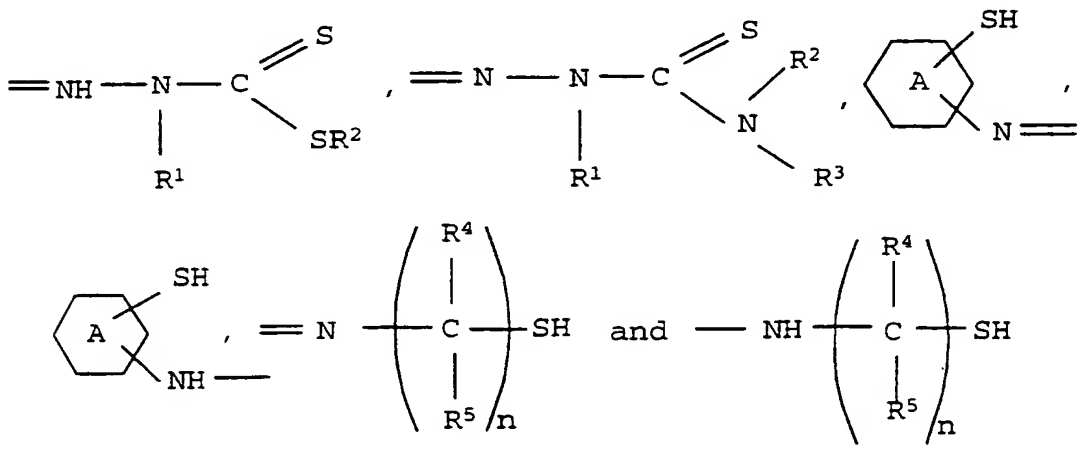
CLAIMS

1. A radiopharmaceutical product comprising a polysaccharide provided with sequestering groups linked to the polysaccharide by covalent bonds and chosen from among the groups of formulae R-NH-, R-N=, and



in which R is a hydrocarbonic or aromatic group comprising at least one atom of sulphur, and R' is an atom of hydrogen or an alkyl or methyl grouping, said sequestering groups forming, together with a radioactive metal chosen from among technetium, rhenium, copper, yttrium, erbium, gallium and samarium, a complex of the chelate type, in which the polysaccharide is in the form of microparticles.

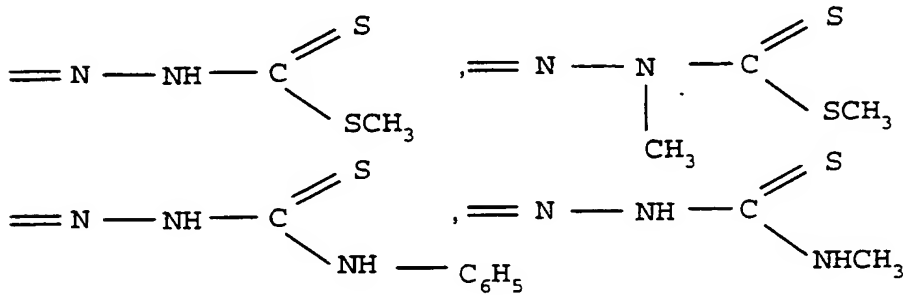
2. A radiopharmaceutical product according to Claim 1 in which the sequestering groups are chosen from among the groups of formulae:

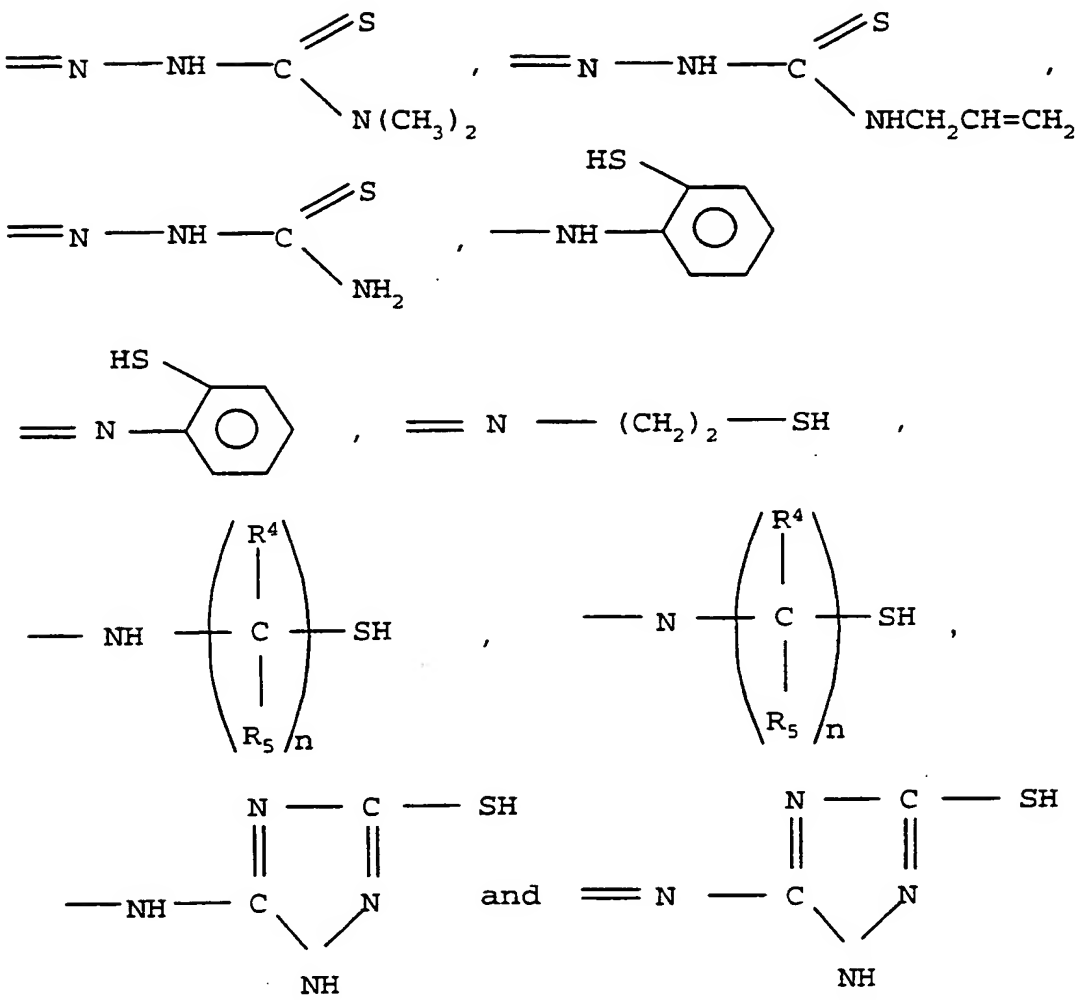


in which R^1, R^2, R^3, R^4 and R^5 are independently atoms of hydrogen atoms, saturated or unsaturated hydrocarbonic groups, carboxylic groups or aromatic groups,

is an aromatic nucleus possibly containing one or several heteroatoms, and n is a whole number between 1 and 5.

3. A radiopharmaceutical product according to Claim 2 in which the sequestering groups are chosen from among the groups of formulae:





4. A radiopharmaceutical product according to any one of Claims 1 to 3, in which the polysaccharide is chosen from among natural starch, cellulose and reticulated amyl pectin.

5. A radiopharmaceutical product according to any one of Claims 1 to 5, in which the microparticles have a dimension between 0.01 and 100 µm

6. A radiopharmaceutical product according to any one of Claims 1 to 5, in which the level of

sequestering groups is from 0.1 to 50% relative to the saccharide patterns of polysaccharide.

7. Utilisation of a radiopharmaceutical product according to any one of Claims 1 to 6, in which the radioactive metal is ^{99m}Tc or ^{67}Ga to prepare a product intended for diagnosis.

8. Utilisation of a radiopharmaceutical product according to any one of Claims 1 to 6, in which the radioactive metal is rhenium-186 or 188, copper-64 or 67, yttrium 90, erbium 169 or samarium 153, to prepare a drug.

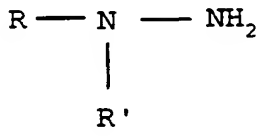
9. Utilisation of a radiopharmaceutical product according to any one of Claims 1 to 7, in which the radioactive metal is ^{99m}Tc to prepare a product intended for pulmonary scintigraphy.

10. A radiopharmaceutical product according to any one of Claims 1 to 6, under the form of a suspension of microspheres in a physiologically acceptable liquid or in lyophilised form.

11. A procedure for preparation of a radiopharmaceutical product according to any one of Claims 1 to 6, which comprises the following stages:

(a) submit a polysaccharide to an oxidation carried out by means of a periodate,

(b) make the oxidated polysaccharide react with a compound containing a primary amine function or hydrazin of formula $R-NH_2$ or

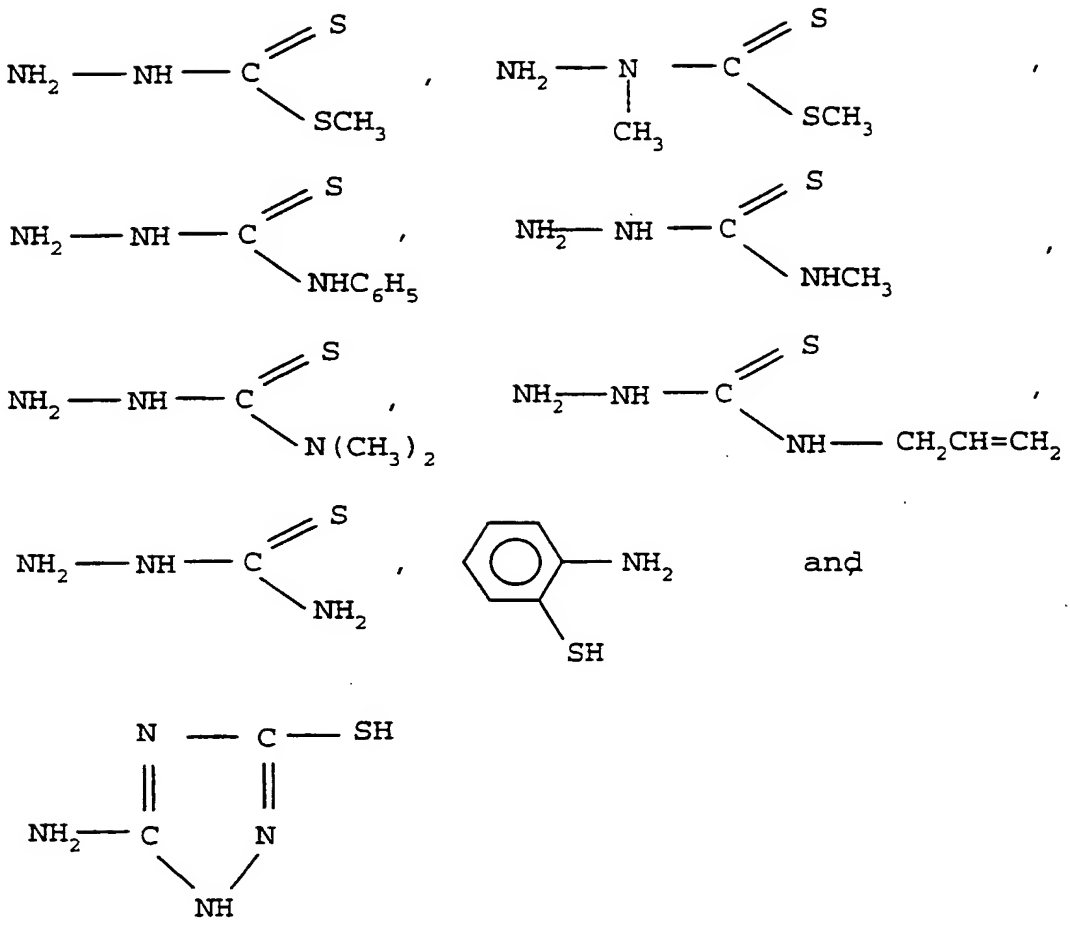


in which R is a hydrocarbonic or aromatic group comprising at least one atom of sulphur, in order to bond in a covalent manner to the polysaccharide with sequestering groups the metals of formulae R-NH-, R-N= or R-NH-N=, and R' is a hydrogen atom or an alkyl or methyl grouping.

(c) make the polysaccharide comprising the sequestering groups react with a salt of a radioactive metal chosen from among technetium, rhenium, copper, yttrium, erbium and samarium.

12. A procedure according to Claim 11, in which the compound containing a primary amine function corresponds to the formula $NH_2-(CH_2)n-SH$ with n being a whole number from 1 to 5, and comprising a supplementary stage of reduction of this compound by sodium borohydride between stages (b) and (c).

13. A procedure according to Claim 11, in which the compound bonded to the oxidised polysaccharide corresponds to one of the following formulae:



14. A procedure according to any one of Claims 11 to 13, in which the level of sequestering groups fixed on the polysaccharide is regulated by controlling the level of oxidation of the polysaccharide in stage (a).

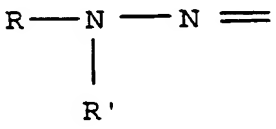
15. A procedure according to Claim 14, in which the oxidation level of the polysaccharide is from 10 to 50%.

16. A procedure according to Claim 14, in which the level of sequestering groups is from 2 to 15%.

17. A procedure according to any one of Claims 10 to 16, in which the stage (c) consists of putting into contact microspheres of polysaccharide comprising sequestering groups with a solution of pertechnetate $^{99m}TcO_4^-$, in the presence of a reducing agent.

18. A diagnosis kit which can be used for pulmonary scintigraphy which comprises:

a first flask containing a polysaccharide provided with sequestering groups linked to said polysaccharide by covalent bonds and chosen among the formulae groups R-NH-, R-N= and



in which R is a hydrocarbonic or aromatic group comprising at least one atom of sulphur, and in which R' is an atom of hydrogen or an alkyl or methyl grouping, in which the polysaccharide is in the form of lyophilised microparticles or in suspension in a pharmaceutically acceptable liquid.

19. A kit according to Claim 18 comprising also a second flask containing stannous chloride in lyophilised form.

20. A kit according to Claim 18, in which the polysaccharide being in the form of lyophilised

microparticles in the first flask, said first flask also contains lyophilised stannous chloride.